The Papers

Long-term temporal trends in metabolic risk factors

Langfristige Trends bei metabolischen Risikofaktoren

Abstract

Objectives: The "metabolic syndrome" characterized by obesity, hypertension, dyslipidemia and hyperglycemia has consistently been associated with increased risk of cardiovascular disease. The aim of this study was to investigate long-term trends in major metabolic risk factors in three large cohorts.

Materials and Methods: Data from 239,602 individuals aged 25-64 years participating in health examinations from 1976 to 2005 were used to estimate prevalence and trends in risk factors.

Results: Irrespective of geographic location, single components of the metabolic syndrome showed divergent trends across the observation period. Whereas obesity and hyperglycemia increased by a per decade ratio of 1.54 (95%CI: 1.42-1.66) and 1.62 (95%CI: 1.49-1.76) in men and 1.48 (95%CI: 1.41-1.56) and 1.66 (95%CI: 1.57-1.75) in women, respectively, hypertension, decreased by 0.71 (95%CI: 0.68-0.74) in men and 0.83 (95%CI: 0.79-0.86 in women. Dyslipidemia showed non-linear pattern. Metabolic syndrome, defined as a combination of three of these risk factors, significantly increased by a ratio of 1.15 (95%CI: 1.08-1.22) and 1.20 (95%CI: 1.15-1.27) per decade in men and women, respectively.

Conclusion: This study showed that the single metabolic risk factors show divergent trends over the period of three decades even if Mets appeared to be stable over the last two decades. The two key components of the syndrome namely BMI and glucose levels, increased significantly deserving professional attention.

Key words: metabolic syndrome, body mass index, blood pressure, blood lipids, blood sugar

Introduction

The "metabolic syndrome" (MetS) was introduced in 1988 as a constellation of metabolic aberrations caused by overeating, sedentary life-style and other predispositions. Since its initial description, several definitions have been proposed [1, 2]. Despite differences in specific criteria among these definitions, there is wide agreement that the major characteristics of the syndrome include obesity, insulin resistance, hyperinsulinaemia, hyperglycaemia, high blood pressure, and dyslipidaemia. Presence of the MetS has consistently been associated with increased risk of cardiovascular disease, stroke and diabetes [3, 4]. There is also a growing body of literature indicating metabolic factors as risk elements for cancer incidence and mortality [5-12].

Epidemiologic evidence regarding long-term trends of single metabolic risk factors is limited. Trends of increasing BMI were consistently reported in studies covering a majority of European countries including the World Health Organisation (WHO) MONICA project [13-17] as well as the U.S. population [18-21] e.g. the NHANES surveys. More divergent results were published concerning blood pressure, cholesterol and triglycerides. The majority of studies showed a decreasing trend in blood pressure [13, 20-21], however a few studies have detected increasing trends [16-17,19]. Inconsistent trends were also observed for cholesterol [14-17,21] and triglycerides [14,19]. Smoking, not a metabolic but a major risk factor for cardiovascular and cancer disease, decreased especially in men in all western industrialized countries [14,20].

Differences in the compositions of the populations studied, limited length of observation periods and insufficient sample size may have contributed to a lack of consistent evidence in previous investigations. Based on the large sample size and an observation period covering 30 years, we aim to analyse if temporal trends in metabolic risk factors exist in different cohorts and health surveys across middle and northern Europe.

Material and Methods

The study population

The Me-Can collaborative study (Metabolic syndrome and Cancer project) was initiated in 2006, in order to create a large pooled cohort to investigate factors of the metabolic syndrome on the association with cancer risk. We used three sub-cohorts of the Me-Can collaborative study, mentioned below, from Austria and Sweden with total participants of 122,076 men and 117,526 women in the age range of 25-64 years. Whereas data from VHM&PP were used to show risk factor prevalence for Austria, VIP and Malmö provided data for Sweden. Although not necessarily representative for the whole country we merged data from Malmö and VIP in order to cover three decades of risk factor data for Sweden.

The Vorarlberg Health Monitoring and Prevention Programme (VHM&PP)

The VHM&PP is a population-based risk factor surveillance programme in Vorarlberg, the westernmost province in Austria [22-23]. The purpose of the programme was to prevent chronic diseases, foremost cardiovascular diseases and cancer, and it was routinely performed by the Agency for Social and Preventive Medicine. All adult residents in the region were invited by written invitations, television, radio or news papers, to participate in a health examination up to once a year. Since 1985, more than two-thirds of the population of the province participated in the programme. Data from the years 1985-2005 is included in Me-Can and during these years, the attendance rate in the VHM&PP was 66% and roughly 176 000 persons participated in the programme.

The Västerbotten Intervention Project (VIP)

The Västerbotten Intervention Project (VIP) is an ongoing project aiming for prevention of diabetes and cardiovascular disease in residents of Västerbotten county in the north of Sweden [24]. Since 1985, all residents are invited for a health check-up at 40, 50 and 60 years of age, and during the first ten years of the project, residents were also invited at the age of 30. The attendance rate has been 60% on average over the years. By the end of 2006, approximately 86,000 men and women had participated in the VIP.

The Malmö Preventive Project (MPP)

Middle-aged men and women in the city of Malmö in southern Sweden, were invited to a screening programme for prevention of cardiovascular disease and alcohol abuse.[25,26] Screenings were carried out between the years 1974-1992, to which all residents within predefined birth cohorts, born between 1921-1949, were invited. The attendance rate was on average 71% over the years. A total of 33,346 men and women participated in the baseline screening, and 5722 of these men (born 1926-1938) and 387 women (born in 1931) participated in a second screening in 1981-1989. The examination at the second screening was similar to that of the first screening.

Ethical approval

The study has been approved by ethical committees of the respective countries.

Measurements

Measurements include height, weight, systolic and diastolic blood pressure, serum total cholesterol, triglycerides, and glucose levels. Anthropometric measurements were conducted in

all cohorts in a similar manner with participants wearing light indoor clothes and no shoes. Blood pressure was measured after 5-10 minutes of rest in sitting position in the VHM&PP and supine position in the VIP the MPP. Measurement was done with mercury sphygmomanometer in all cohorts. Serum measurements were performed after an overnight-fast of at least 8 hours in all individuals. Determination of glucose was done on serum in all cohorts except in MPP, where it was measured in whole blood. For determination of lipids, serum samples were used in all the cohorts. All the analysis was done using enzymatic technique.

The metabolic syndrome

To define the metabolic syndrome, we used a modified form of the National Cholesterol Education Programme Adult Treatment Panel (NCEP ATP III) [27] definition based on five commonly measured criteria: waist circumference (WC) which we replaced by BMI, blood pressure, serum triglycerides, serum high-density lipoprotein (HDL) cholesterol and fasting glucose level. In our study total cholesterol measurements were available instead of HDL-cholesterol. We used the following cut-off points: BMI $\geq$ 30 kg/m^2, hypertension defined as systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg, hypercholesterolemia as total cholesterol > 6.1 mmol/l, hypertriglyceridaemia as triglycerides $\geq$ 1.69 mmol/l and fasting hyperglycaemia as fasting glucose $\geq$ 6.1 mmol/l. Any combination of three of the above conditions was defined as metabolic syndrome.

Statistical analysis

Total and region specific prevalence and means of metabolic risk factors were estimated by decades separately for men and women. Trends in metabolic risk factors were assessed by sex-specific linear and logistic regression analyses adjusted for age, region, smoking status and BMI where appropriate. For the logistic regression analyses risk factors were dichotomized in normal and elevated levels according to standard guidelines as reported above. The surveys included few participants above 65 years of age. Therefore, we restricted our analysis to the age range of 25-64 years. However, to further achieve balanced participation conform to the known population age distribution in this age range we applied sampling weights for the different cohorts using data from official census for the respective countries in the respective decade [28,29]. Sampling weights were calculated for 5-year age groups, separately for sex, decade and region. Results were summarized according to decades and the following regions: Austria (VHM&PP), and Sweden (VIP, MPP). SPSS Complex Samples 16.0 (SPSS Inc. Chicago, Il 2007) was used for statistical analysis.

Results

Estimated prevalence of the Mets and its components divided by regions and decades are shown in Table 2 for men and Table 3 for women. Mets defined as any combination of three elevated single risk factors increased from 10.3% to 15.8% in men and from 4.4% to 10.1% in women from the first to the second decade, followed by a very slight decrease from the second to the third decade (14.2% in men, 9.9% in women). Overall, prevalence of Mets increased by a ratio of 1.15 (95% CI: 1.08-1.22) and 1.20 (95% CI: 1.15-1.27) per decade, in men and women, respectively.

The single risk factors showed divergent trends across the three decades in both sexes (Tables 2, 3). The predominant trend was that obesity and impaired fasting glucose showed a strong increasing pattern whereas blood pressure lowered over the decades in both men and women. Dyslipidemia showed a less clear pattern. The prevalence of both hypercholesterolemia and hypertriglyceridemia increased from the first to the second decade and decreased from the second to the third. For hypercholesterolemia, values were significantly lower in the most recent decade; in the case of hypertriglyceridemia changes were not significant. Prevalence of smoking declined markedly in both men and women over the three decades. Regarding risk factor trends in the sub-regions, the observed results are similar to the overall results, with the exception of hypertension that decreased less pronounced in the Austrian survey compared to the Swedish surveys.

Trends in estimated means roughly confirmed the results of categorised risk factors. As shown in Tables 4 and 5 mean BMI and fasting glucose values for both regions increased significantly whereas diastolic blood pressure decreased and average cholesterol level remained stable. However, mean systolic blood pressure did not change significantly pointing to the possibility that the decrease in the prevalence of hypertension over the years is mainly due to control of diastolic blood pressure.

Discussion

The results of this analysis of three large-scale cohort datasets in two European countries provide further consistent evidence that metabolic risk factors followed divergent patterns across the past three decades. Whereas BMI and glucose levels increased uniformly, blood pressure and smoking decreased. Trends in blood lipids followed a non-linear pattern. Our findings concerning obesity are in agreement with all major studies performed in western industrialized countries [15-16, 30-31]. Similar obesity prevalence was reported for the European countries of the WHO MONICA project, albeit spanning a shorter time-period [15]. Whilst the magnitude of the increase of prevalence is markedly higher in the US, where the NHANES surveys showed that the age adjusted prevalence of obesity (BMI$\geq$ 30kg/m^2) rose from 29.1% in 1960-62 to 49.8% in 1999-2000 among the Afro-Americans and from 12.3% to 35.7% among Caucasians in the respective years [32], the direction of the trends are similar to our findings.

Our results for glucose, blood pressure and total cholesterol were also consistent with the reports from MONICA and NHANES. It has been argued recently that the forces driving these trends operate at the population rather than the individual level because this occurs across the percentile distribution [14, 31] and this between-country evidence we present here further supports that contention. Regarding hypertension, however, the few studies performed report conflicting outcomes [16, 20, 33]. This is a risk factor that can be strongly dependent on clinical hypertension detection and treatment follow-up and we demonstrate some differences between countries and cohorts that suggest this may indeed be important. Moreover, the zero-five-end digit preference might have contributed to the increase in the likelihood of classifying individuals as hypertensive as also seen else where [34]. Additionally it is suggested that both the baseline systolic and diastolic blood pressures were significantly higher in sitting position in both volunteers and hypertensive patients as compared to supine position [35]. This could, partly, be

the case in our study where by the blood pressure values are higher in the Austrian cohorts as compared to the Swedish.

The pattern of lipid levels over time was not linear, and similarly, a previous study from Sweden showed an increase between 1985 and 1995, followed by a decline between 1995 and 2002 [15]. Prevalence of the MetS is strongly influenced by the different definitions proposed by the WHO, the NCEP or the IDF (International Diabetic Federation) [27]. Definitions overweighing glucose lead to increasing trends whereas other definitions show stable or even decreasing trends. Notably, whilst some risk factor trends are downwards and some conversely upwards, the net pattern for presence of three or more metabolic risk factors has in effect plateaued in the last decade. But the overall increasing tendency of metabolic syndrome was also observed in the NHANES surveys among US adults which also applied the ATPIII definition [19].

The implications of this may signal no net change in incidence of cardiovascular disease but a differing clinical presentation, whereas for cancer the implications may be quite different, especially given the strongly demonstrated association between obesity and cancer outcome in recent reports. Obesity was repeatedly shown to have positive association with overall and several site-specific malignancies including cancers of colorectum, endometrium, pancreas, kidney, gallbladder, thyroid and oesophagus [6-8]. Furthermore, obesity was shown to be associated with high prostate and breast cancer mortality [9, 10]. High glucose levels were also linked to an increased overall risk of cancer [11-12].

Our study had several strengths and potential limitations that should be considered. Major strengths are the large sample size, length of observation period and the comparable protocols between middle and northern European health surveys that were used assessing the data. Measurements were in general very similar, but there were some exceptions. Blood sample for glucose determination was serum in VHM&PP and in VIP and whole blood in MPP.

Additionally, blood pressure was measured in sitting position in VHM&PP and in supine position in VIP and MPP. These may well affect our results, however, we think to a minor extent. A further limitation of the study is the inability to examine the effect of treatment, mainly medication of high blood pressure, cholesterol and diabetes. In addition, we were unable to account for the effect of behavioural changes regarding diet, alcohol consumption or physical activity.

This study showed that the single metabolic risk factors show divergent trends over the period of three decades even if Mets appeared to be stable over the last two decades. The fact that the key components of MetS, namely BMI and glucose levels, increased significantly deserves wide spread recognition by policy makers and those concerned with health promotion and disease prevention.

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Table 1. Sociodemographic characteristics of the study population

Cohort	No. of participants			Mean age (std.)*	
	men	women	total	men	women
VHM&PP (Austria)					
76-85	-	-	-	-	-
86-95	42,263	49,094	91,357	42.6 (11.1)	42.8 (11.3)
96-05	23,054	21,339	44,393	40.7 (11.0)	39.8 (10.9)
Malmö (Sweden)					
76-85	22,241	6,750	28,991	43.7 (6.6)	46.6 (7.7)
86-95	-	3,774	3,774	-	54.8 (2.1)
96-05	-	-		-	
VIP (Sweden)					
76-85	-	-	-	-	-
86-95	12,597	14,194	26,791	45.4 (10.4)	45.5 (10.4)
96-05	21,921	22,375	44,296	48.8 (8.4)	48.7 (8.5)

* Only participants between 25 and 64 years of age were included.

Table 2. Total and region-specific prevalence of metabolic risk factors and smoking by decades in men aged 25-64 years, Austria, Sweden 1976-2005

No. of participants	decades	obesity*	hypertension	hypercholeste-rolemia	hypertrigly-ceridemia	fasting hyperglycemia	3 or more factors (modified ATP)	smoking
Total								
n=22,241	76-85	6.1 †	43.0	27.1	26.2	4.7	10.3	47.9
n=54,860	86-95	9.8	35.5	34.7	35.0	8.7	15.8	24.7
n=44,975	96-05	14.1	33.9	25.4	30.7	13.6	14.2	19.8
	OR (95% CI)	1.54 (1.42-1.66)††	0.71 (0.68-0.74)	0.87 (0.83-0.91)	0.98 (0.94-1.03)	1.62 (1.49-1.76)	1.15 (1.08-1.22)	0.54 (0.52-0.57)
	p for trend	<0.001	<0.001	<0.001	0.278	<0.001	<0.001	<0.001
Austria								
-	76-85	-	-	-	-	-	-	-
n=42,263	86-95	9.7	42.0	34.2	38.7	8.2	16.8	32.2
n=23,054	96-05	14.2	40.5	24.4	34.7	12.6	16.0	25.5
Sweden								
n=22,241	76-85	6.1	43.0	27.1	26.2	4.7	10.3	47.9
n=12,597	86-95	9.8	28.8	34.9	30.1	9.0	13.9	17.8
n=22,921	96-05	13.8	27.2	26.1	26.3	14.2	12.1	14.4

* Obesity was defined as BMI ≥30 kg/m², hypertension as sytolic blood pressure of ≥140 mmHg or diastolic ≥90 mmHg, hypercholesterolemia > 6.1 mmol/l, hypertriglyceridemia ≥1.69 mmol/l and hyperglycemia >6.1 mmol/l.

†Percentages estimated using region-,decade-,and sex-specific 5-year age-group sampling weights (complex samples).

†† Ratio of change per decade, estimated from logistic regression models for complex samples adjusted for age, region, smoking and body-mass-index where appropriate.

Table 3. Total and region-specific prevalence of metabolic risk factors and smoking by decades in women aged 25-64 years, Austria, Sweden 1976-2005

No. of participants	decades	obesity*	hypertension	hypercholeste-rolemia	hypertrigly-ceridemia	fasting hyperglycemia	3 or more factors (modified ATP)	smoking
Total								
n=6,750	76-85	6.6 †	25.4	24.2	11.8	1.8	4.4	39.7
n=67,062	86-95	11.4	27.7	28.0	17.4	6.4	10.1	23.2
n=43,714	96-05	15.6	26.5	22.6	15.7	9.3	9.9	17.9
	OR (95% CI)	1.48 (1.41-1.56)††	0.83 (0.79-0-86)	0.82 (0.80-0.86)	0.99 (0.94-1.03)	1.66 (1.57-1.75)	1.20 (1.15-1.27)	0.61 (0.59-0.63)
	p for trend	<0.001	<0.001	<0.001	0.439	<0.001	<0.001	<0.001
Austria								
-	76-85	-	-	-	-	-	-	-
n=49,094	86-95	11.4	33.3	27.6	18.2	6.4	10.9	23.5
n=21,339	96-05	16.3	30.5	21.2	16.7	8.5	10.6	18.4
Sweden								
n=6,750	76-85	6.6	25.4	24.2	11.8	1.8	4.4	39.7
n=17,968	86-95	11.0	20.9	28.2	16.0	6.2	8.6	23.4
n=22,375	96-05	14.5	21.7	23.3	14.4	9.8	8.7	17.7

* Obesity was defined as BMI ≥30 kg/m², hypertension as sytolic blood pressure of ≥140 mmHg or diastolic ≥90 mmHg, hypercholesterolemia > 6.1 mmol/l, hypertriglyceridemia ≥1.69 mmol/l and hyperglycemia >6.1 mmol/l.

†Percentages estimated using region-,decade-,and sex-specific 5-year age-group sampling weights (complex samples).

†† Ratio of change per decade, estimated from logistic regression models for complex samples adjusted for age, region, smoking and body-mass-index where appropriate.

Table 4. Total and region-specific means (95% CI) of metabolic risk factors by decades in men aged 25-64 years, Austria, Sweden 1976-2005

cohort	decades†	BMI, kg/sqm	systolic blood pressure, mmHg	diastolic blood pressure, mmHg	total cholesterol, mmol/l	triglycerides, mmol/l	fasting glucose, mmol/l
Total							
n=22,241	76-85	24.5 (24.4-24.7)*	128.4 (127.7-129.0)	85.0 (84.7-85.5)	5.6 (5.5-5.6)	1.5 (1.4-1.5)††	5.1 (5.0-5.2)
n=54,860	86-95	25.6 (25.5-25.6)	129.7 (129.5-129.9)	81.1 (81.0-81.2)	5.7 (5.7-5.7)	1.7 (1.7-1.7)	5.1 (5.1-5.1)
n=44,975	96-05	26.2 (26.1-26.3)	129.9 (129.6-130.1)	80.6 (80.4-80-8)	5.5 (5.5-5.5)	1.6 (1.6-1.6)	5.4 (5.4-5.4)
	p for trend	<0.001†	0,011	<0.001	<0.001	0.24	<0.001
Austria							
-	76-85	-	-	-	-	-	-
n=42,263	86-95	25.5 (25.4-25.5)	131.4 (131.2-131.6)	82.5 (82.4-82.6)	5.7 (5.7-5.7)	1.8 (1.8-1.8)	4.8 (4.8-4.8)
n=23,054	96-05	26.0 (26.0-26.1)	132.2 (131.9-132.5)	82.2 (82.0-82.3)	5.6 (5.6-5.6)	1.7 (1.7-1.7)	5.3 (5.2-5.3)
Sweden							
n=22,241	76-85	24.5 (24.4-24.7)	128.4 (127.5-128-2)	85.1 (84.7-85.5)	5.6 (5.5-5.6)	1.5 (1.4-1.5)	5.1 (5.0-5.2)
n=12,597	86-95	25.7 (25.6-25-7)	127.8 (127.5-128.2)	79.7 (79.5-80.0)	5.7 (5.7-5.7)	1.5 (1.5-1.6)	5.3 (5.3-5.4)
n=22,921	96-05	26.3 (26.2-26.4)	127.5 (127.1-127.9)	79.1 (78.8-79.3)	5.4 (5.4-5.5)	1.5 (1.4-1.5)	5.5 (5.5-5.6)

*Means (95% CI) estimated using region-, decade-, and sex-specific 5-year age-group sampling weights (complex samples).

† Effect of decade (p for trend) was tested in linear regression analyses for complex samples adjusted for age, region, smoking and body-mass-index where appropriate. In case of non linear pattern (lipids) p-values were given comparing 3rd versus 1st decade.

†† Triglycerides were log-transformed, means thus obtained represent geometric means.

Table 5. Total and region-specific means (95% CI) of metabolic risk factors by decades in women aged 25-64 years, Austria, Sweden 1976-2005

cohort	decades†	BMI, kg/sqm	systolic blood pressure, mmHg	diastolic blood pressure, mmHg	total cholesterol, mmol/l	triglycerides, mmol/l	fasting glucose, mmol/l
Total							
n=6,750	76-85	23.4 (23.2-23.5)*	121.2 (120.7-121.7)	80.6 (80.3-80.9)	5.5 (5.5-5.5)	1.1 (1.1-1.1) ††	4.8 (4.7-4.8)
n=67,062	86-95	24.7 (24.6-24.7)	125.1 (124.9-125.3)	78.3 (78.2-78.4)	5.5 (5.5-5.5)	1.3 (1.2-1.3)	5.0 (5.0-5.0)
n=43,714	96-05	25.3 (25.2-25.4)	125.0 (124.7-125.3)	77.4 (77.2-77-6)	5.4 (5.4-5.4)	1.2 (1.2-1.2)	5.2 (56.2-5.3)
	p for trend	<0.001†	0.512	<0.001	<0.001	0.288	<0.001
Austria							
-	76-85	-	-	-	-	-	-
n=49,094	86-95	24.4 (24.4-24.5)	127.1 (126.9-127.3)	80.0 (79.8-80.1)	5.5 (5.5-5.5)	1.3 (1.3-1.3)	4.7 (4.7-4.7)
n=21,339	96-05	25.1 (25.0-25.2)	126.6 (126.2-126.9)	79.0 (78.8-79.2)	5.4 (5.4-5.5)	1.2 (1.2-1.2)	5.0 (5.0-5.1)
Sweden							
n=6,750	76-85	23.4 (23.2-23.5)	121.2 (120.7-121.7)	80.6 (80.3-80.9)	5.5 (5.5-5.5)	1.1 (1.1-1.1)	4.8 (4.7-4.8)
n=17,968	86-95	24.8 (24.7-24.9)	122.5 (122.2-122.8)	76.5 (76.3-76.7)	5.5 (5.5-5.5)	1.2 (1.2-1.2)	5.2 (5.2-5.2)
n=22,375	96-05	25.4 (25.3-25.6)	123.0 (122.6-123.4)	75.6 (75.3-75.9)	5.4 (5.3-5.4)	1.2 (1.2-1.2)	5.4 (5.4-5.4)

*Means (95% CI) estimated using region-,decade-,and sex-specific 5-year age-group sampling weights (complex samples).

† Effect of decade (p for trend) was tested in linear regression analyses for complex samples adjusted for age, region, smoking and body-mass-index where appropriate. In case of non linear pattern (lipids) p-values were given comparing 3rd versus 1st decade.

†† Triglycerides were log-transformed, means thus obtained represent geometric means.

Serum Triglycerides and Cancer Risk in the Metabolic Syndrome and Cancer (Me-Can) Collaborative Study

Running Title: Serum triglycerides and cancer risk

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Abstract

Objective: To assess the association between serum triglyceride levels and cancer risk.

Methods: The metabolic syndrome and cancer project (Me-Can) includes cohorts from Norway, Austria, and Sweden; the current study included data on 257,585 men and 256,512 women. The mean age at study entry was 43.8 years for men and 44.2 years for women. The mean follow-up time was 13.4 years (SD=8.5) for men and 11.9 years (SD=7.2) for women. Excluding the first year of follow-up, 23,060 men and 15,686 women were diagnosed with cancer. Cox regression models were used to calculate relative risk (RR) of cancer for triglyceride levels in quintiles and as a continuous variable. RRs were corrected for random error by use of regression dilution ratio.

Results: Relative risk for top quintile versus bottom quintile of triglycerides of overall cancer was 1.16 (95% confidence interval 1.06-1.26) in men and 1.15 (1.05-1.27) in women. For specific cancers, significant increases for top quintile versus bottom quintile of triglycerides among men were found for cancers of the colon, respiratory tract, the kidney, melanoma and thyroid and among women, for respiratory, cervical and non-melanoma skin cancers.

Conclusion: Data from our study provided evidence for a possible role of serum triglycerides in cancer development.

Key words: lipids, prospective study, cancer incidence

Introduction

High levels of serum triglycerides (STG) in blood are related with coronary heart disease [1-6], however, the relationship between serum triglycerides and cancer is less well known. Several pathological and molecular mechanisms suggested a link between triglycerides, as part of the metabolic syndrome, and risk of cancer [7-9]. However, prospective studies on the relation of serum triglycerides to overall and site specific cancers are rare [10, 11]. Although a few studies have reported on the association with site-specific cancer such as colorectal, breast and prostate; results are contradictory [12-17].

Differences in the study populations, length of follow-up, study endpoints and statistical adjustment for confounding may all have contributed to the conflicting patterns of association seen in earlier studies. Consistent results may also be lacking due to small sample sizes and infrequent events in several previous investigations. Moreover, the role of regression dilution bias in long-term prospective studies of incident diseases in relation to baseline risk factors measured at single occasion was not considered before [18].

The aim of the study was to investigate the association between serum triglycerides and overall and site-specific cancer risk in a large prospective cohort of 514,097 participants with 38,746 incident cancers. To our knowledge, this is the largest cohort study to date to prospectively investigate the association of STG with overall and site-specific cancer risk in men and women.

Materials and Methods

Study population

The Metabolic syndrome and Cancer project (Me-Can) includes data from population-based cohorts in Norway, Austria, and Sweden. A detailed description of the project has recently been published [19, 20]. In brief, seven existing cohorts from Austria (the Vorarlberg Health Monitoring and Prevention Programme-VHM&PP), Norway (the Oslo study, the Norwegian Counties study-NCS, the Cohort of Norway-CONOR and the Age 40 programme-40-y) and Sweden (the Västerbotten Intervention Project-VIP and the Malmö Preventive Project-MPP) were pooled in 2006. Participants in the cohorts had undergone one or more health examination(s), and information on life style and socio-demographic factors had been recorded. Altogether, data on 514,097 men and women were used in this study.

Measurements

Measurements of height, weight, smoking status (current, former, never), serum triglycerides have been obtained for the study participants in various health examinations. Anthropometric measurements were conducted in a similar way in all Me-Can cohorts, with participants wearing light indoor clothes and no shoes. For smoking status participants (except in VHM&PP) were asked to fill in a questionnaire regarding smoking habits. In VHM&PP, questions regarding smoking were asked by the examining physician, and answers were recorded.

Different fasting times before blood draw were used in the different cohorts [19]. In the Norwegian cohorts, fasting was not required before the examination, and fasting time was recorded as less than 1 hour, 1-2, 2-4, 4-8, or more than 8 hours. Fasting time in the VIP was recorded as less than 4 hours, 4-8, or more than 8 hours, and

from 1992, participants were asked to fast for at least eight hours before the examination. In the MPP and after the initial three years in the VHM&PP, a minimum of eight hours of fasting was used as standard procedure. Serum levels of triglycerides were measured with a non-enzymatic method in the Oslo and NCS cohorts, and with an enzymatic method in all other cohorts. Levels measured with the non-enzymatic method have been transformed according to formulas: 0.90 (triglyceride level)–0.11 and are presented in mmol/L [19].

Selection of subjects

The full Me-Can dataset included 924,801 participants with 1,566,553 health examinations. We selected the first observation for each individual, and if data from a fasting state and data on smoking status were available, the first of these observations was selected. Thus, for each individual, data were included from the first health examination with complete data to comprise the baseline set of measurements. Subjects were further excluded from the Norwegian cohorts due to policy restrictions imposed by the Norwegian Institute of Public Health that the proportion of Norwegian subjects in Me-Can studies should not exceed approximately 50%. This was done by selecting all the subjects who have fasted >8 hours in one of the sub-cohorts (40 year cohort) and randomly selecting 39,673 non-fasting subjects from the same sub-cohort. Exclusions were made for observations with cancer diagnosis before the date of baseline examination, and for time less than 1 year after measurement in order to avoid the effect of short term reverse causation. Further exclusions were made for observations without data on fasting time and observations with fasting time 4-8 hours. The latter is based on reports showing that triglycerides reach their peak postprandial level within 4 hours of ingestion and clearance from serum occurs slowly reaching a fasting state 8-12 hours after

ingestion [3, 21]. The final data set used in this study included 514,097 subjects, 257,585 (50.1%) men and 256,512 (49.9%) women, of which 270,727 (52.7%) had fasted more than 8 hours and 243,370 (47.3%) had fasted for less than 4 hours before the blood sampling.

End points

Each of the cohorts was linked to the respective National registers for identification of cancer diagnosis, emigration, vital status (except for Austria) and cause of death. Incident cancer cases were identified through linkages with national cancer registries of the respective countries. End-points were the date of the first cancer diagnosis, emigration, death or December 31, 2003 (Austria), 2005 (Norway) and 2006 (Sweden), whichever occurred first. Cancers were categorized according to the International Classification of Diseases, seventh revision (ICD-7).

Statistical analysis

We used Cox proportional hazard regression to estimate hazard ratios, denoted as relative risks (RR), for triglyceride levels with risk of incident cancer. Age was used as time variable and all estimates were stratified by sub-cohort and categories of birth date: ≤ 1929, 1930-1939, 1940-1949, 1950-1959 and ≥ 1960. We estimated RR for triglycerides levels in quintiles calculating cut-off levels separately for sub cohort, sex, and fasting time. Linear tests for trend were performed using triglyceride quintiles as an ordinal variable. Secondary analysis was done using triglycerides as a logarithmically transformed (due to the skewed distribution) continuous variable. All analyses included adjustment for age at baseline measurement (continuous), BMI (categories: $\leq$20.0, 20.01-, 25.01-, 30.01 kg/m^2) and smoking status (categories: never smoker, ex-smoker, and current smoker). All models were further adjusted for fasting time in the secondary analysis where triglycerides were used as continuous variable. Lag-time analyses leaving out the first 5 and 10 years of follow-up were performed to check for reverse causation effects.

We calculated regression dilution ratio (RDR) in order to adjust RRs for random measurement error [22-23]. RDR was calculated based on data from repeated health examinations in 133,820 subjects, including 406,364 observations, in the full Me-Can cohort. Only repeated measurements with the same fasting time and in the same cohort as in the baseline, and with data on smoking status, were used. Mean time between the baseline measurement and repeated measurements was 6.9 years (standard deviation, SD = 3.9). We used a linear mixed effects model, similar to that described by Wood et al [23].

We checked the Cox proportional hazards assumption for triglycerides, BMI, and smoking status by the statistical test of Schoenfeld residuals. For some cancers, there was an indication of violation of proportionality for BMI or smoking status;

however, RRs with and without stratification of the variable within the model were very similar. Thus, BMI and smoking status were not kept as stratum in the model. Statistical analyses were performed with Stata (version 10, StataCorp LP, College Station, Texas), and R (version 2.7.2, used for RDR calculation).

Results

The mean age at study entry was 43.8 years for men and 44.2 years for women. The mean follow-up time was 13.4 years (SD=8.5) for men and 11.9 years (SD=7.2) for women. (Table 1) During follow-up, 38,746 incident invasive cancer cases were identified. Baseline age, BMI and total cancer risk increased with higher triglyceride quintiles.

Table 2 shows association of STG with cancer risk in men. Compared with the 1st quintile, STG concentrations in 5th quintile were significantly associated with increased risk of total cancer (RR: 1.16; 95% CI, 1.06-1.26), colon cancer (RR: 1.96, 95% CI, 1.44-2.67), cancer of the respiratory system (RR: 1.42; 95% CI, 1.12-1.80), renal cell carcinoma (RR: 1.85, 95% CI, 1.14-3.02), melanoma of the skin (RR: 1.49; 95% CI, 1.00-2.20), and thyroid cancer (RR: 3.49; 95% CI, 1.04-11.57). In contrast, high STG concentrations were inversely associated with risk prostate cancer (RR: 0.83, 95% CI, 0.72-0.98) and Non-Hodgkin's lymphoma (RR: 0.61, 95% CI, 0.39-0.96).

In Table 3, the associations of STG with overall and site-specific cancer risk are shown for women. Relative risk for 5^{th} quintile, as compared to the 1^{st} quintile, were associated with increased risk of total cancer (RR: 1.15; 95% CI, 1.05-1.27), cancer of the respiratory system (RR: 2.10; 95% CI, 1.41-3.12), cervical cancer (RR: 1.88; 95% CI, 1.07-3.30), non-melanoma skin cancer (RR: 1.98; 95% CI, 1.04-3.84) and other non-specified cancers (RR: 1.63; 95% CI, 1.04-2.55).

Furthermore, a positive association per log-unit increment of STG was seen for lip, oral cavity and pharyngeal cancer in both men and women, for stomach and bladder cancers in men and for rectum/anus and brain/nervous tissue cancers in women (Tables 2 and 3).

206 Lag-time analysis leaving out the first 5 and 10 years of follow-up (data not shown)
207 showed that the significant inverse association observed between STG and prostate
208 cancer or non-Hodgkin's lymphoma disappeared.
209 Separate sub-analyses for fasting and non-fasting individuals revealed only a few
210 important differences in comparison to the main analysis. The association of STG
211 with colon cancer in men was more pronounced in fasting individuals showing a
212 relative risk of (RR: 2.70; 95% CI, 1.63-4.53) as compared to non fasting individuals
213 (RR: 1.63; 95% CI, 1.12-2.40). In women, we observed a significant association for
214 non-melanoma skin cancer of (RR: 4.31; 95% CI, 1.56-11.9) versus (RR: 0.96; 95%
215 CI, 0.40-2.36) and a borderline significant effect for corpus uteri of (RR: 1.74; 95%
216 CI, 0.99-3.09) versus (RR: 1.07; 95% CI, 0.65-1.72) for fasting versus non-fasting
217 STG respectively. In contrast, only non-fasting STG showed a significant association
218 with renal cell cancer (RR: 2.23; 95% CI, 1.14-4.37) versus (RR: 1.42; 95% CI, 0.72-
219 2.83) and thyroid cancer (RR: 6.60; 95% CI, 1.40-30.66) versus (RR: 1.24; 95% CI,
220 0.17-8,47) in men and with cervical cancer (RR: 2.64; 95% CI, 1.29-5.52) versus
221 (RR: 1.04; 95% CI, 0.37-2,77) in women.
222

Discussion

In this pooled population-based cohort study of more than 500,000 participants, elevated serum triglyceride levels were associated with significantly increased risk of cancer in general and at several specific sites. The relative risk of total cancer for increasing levels of triglycerides on overall cancer, observed both in men and women, was a modest one, but for several specific sites the increases in relative risk was quite formidable. Although our study is the first to show significant association between STG and overall cancer in men and women separately, some of our results of site specific cancers are in accordance with the other previous prospective studies [11, 12, 24]. However, in these studies, regression dilution bias, caused by random fluctuations in baseline measurements in long-term prospective studies, was not accounted for and thus, existing random error might have underestimated the true risk. Long-term effects of triglyceride levels on coronary heart disease occurring more than 20 years after the baseline measurement have been reported to be underestimated by 50% [18]. Our study is the first to consider the possible role of regression dilution in estimating cancer risk in relation to serum triglyceride levels.

We observed a comparatively strong positive association between STG levels and colon cancer in men. Although several previous studies investigated this association, results have been inconsistent [10, 12, 13, 24]. Several reports suggested positive associations between triglycerides and obesity in relation to colorectal cancer indicating possible confounding effects [7, 8, 12]. In our study, however, the models were adjusted for BMI. Thus significant association shows a strong link between STG and colon cancer. This association was not seen among women speculatively due to a protective effect of estrogen and progesterone on colon cancer [25, 26].

We also observed an association between STG and increased risk of cancers of the respiratory tract among men and women. We checked for possible interaction

between smoking and serum triglycerides (data not shown); but no significant interaction was observed. The association of STG and respiratory cancer may possibly be related to the fact that smoking is associated with higher STG concentrations [27], and thus, residual confounding due to smoking may contribute to the association between STG concentrations and lung cancer risk as well as cancers of lip, oral cavity and pharynx.

The significant positive association of serum triglycerides with cervical cancer was also observed in other prospective studies [10, 11]. According to Cowey et al, increased levels of free fatty acids (FFAs) may be associated with reduced production of sex hormone binding globulins (SHBGs) by the liver. Low levels of SHBGs are associated with increased availability of bioactive estradiol (E2) which, in turn are considered to be mitogenic growth factors for some hormone dependent cancers [9].

Although transformation of androgens to estrogens in obese men might be a possible explanation for the inverse association between STG and prostate cancer [9], cohort studies assessing metabolic syndrome and triglycerides in relation to prostate cancer so far have not shown any significant association [16, 17]. Moreover, we cannot rule out a possible reverse causality as the significance of the association disappeared in a sub analysis that excluded the first 5 years of follow-up.

Other possible mechanistic evidence relating elevated triglycerides with cancer risk is the development of oxidative stress and reactive oxygen species (ROS). Low levels of ROS regulate cellular signaling and play an important role in normal cell proliferation, and are increased in cancer cells. [28,29]. Furthermore, it is suggested that ROS react with fatty acid components of triglycerides producing a chemical substance called bifunctional aldahyde which, in turn, is associated with gene mutation [9].

Strengths of our study include the large sample size from seven population-based cohorts in Europe with essentially complete capture of cancer cases and the correction of risk estimates for intra-individual variation of triglyceride levels. In all cohorts, data were available for BMI and smoking status, and these factors were used as adjustment in the analyses. The long follow up period provided the opportunity to undertake lag-time sub analysis helping to rule out possible reverse causalities. However, our study was limited by the lack of data on drug use and other behavioral aspects like dietary habits, physical activity and alcohol consumption which could be associated with triglycerides [30].

A report on the role of fasting versus non-fasting serum triglyceride measurement suggested that collection of specimen at a specified postprandial state (2-4hours) could be more predictive of cardiovascular risk than in the fasting state [3, 31]. It is not known if the same implication goes for risk of cancer. Although our study showed differences on cancer risk across fasting status, it is difficult to draw conclusions about these differences based on the respective fasting time as the fasting status of the participants in this study was non-uniform among the sub-cohorts, However, these differences are not ignorable and indicate the need to assess the situation in a data where fasting status is presented in unbiased manner.

In summary, in this pooled study of 514,097 men and women, we found significant associations between serum triglycerides and risk of cancer overall and at several sites. Our data provide further evidence for an association between adverse metabolic factors and risk of cancer. Since many of the determinants of high triglycerides levels are known, our data indicate that control of triglyceride levels by a healthy diet and physical activity may decrease risk of cancer at many sites in addition to a decreased risk of cardiovascular diseases.

Conflict of interest

None declared

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Table 1. Baseline characteristics of the study population across quintiles of triglycerides in men and women

		Quintile 1-5				
Men	All men	1	2	3	4	5
	(n=257,585)	(n=52,469)	(n=51,548)	(n=51,247)	(n=51,178)	(n=51,143)
Age, mean±SD , y	43.8 ± 11.7	41.7±12.1	43.5±12.1	44.3±11.9	44.9±11.5	45.0±10.5
Current smoking - no.(%)	92,877 (36.1)	15,925 (30.4)	17,757 (34.5)	18,647 (36.5)	19,490 (38.1)	21,058 (41,2)
BMI, mean±SD (median), kg/m²	25.5±3.5 (25.2)	24.0±2.9 (23.8)	24.7±3.1 (24.5)	25.4±3.3 (25.1)	26.2±3.4 (25.9)	27.4±3.4 (25.9)
Triglycerides, median (IQR), mmol/L	1.51 (1.05-2.22)	0.80 (0.68-0.90)	1.14 (1.00-1.32)	1.51 (1.31-1.74)	2.04 (1.76-2.30)	3.23 (2.74-4.08)
Total cancer risk - no. (%)	23,060 (8.9)	3,944 (7.5)	4,665 (9.0)	4,710 (9.2)	4,971 (9.1)	4,770 (9.3)
Follow-up, mean±SD (median), y	13.4±8.5 (11.2)	13.5±8.7 (10.9)	13.6±8.7 (11.2)	13.5±8.5 (11.3)	13.4±8.4 (11.3)	13.2±8.2 (11.1)
Person years at risk	3,451,639	708,332	701,053	691,835	685,785	675,088
Women	All women	1	2	3	4	5
	(n=256,512)	(n=54,736)	(n=48,967)	(n=51,394)	(n=50,568)	(n=50,847)
Age, mean±SD , y	44.2 ± 13.1	40.0±10.5	42.1±12.1	44.2±13.1	45,9±13.8	49.0±14.0
Current smoking - no.(%)	72,714 (28.4)	12,052 (22.1)	13,458 (27.5)	15,062 (29.3)	15,741 (31.2)	16,401 (32.3)
BMI, mean±SD (median), kg/m²	24.8±4.4 (23.9)	23.0±3.3 (22.5)	23.7±3.7 (23.1)	24.5±4.2 (23.8)	25.5±4.6 (24.8)	27.3±5.0 (26.6)
Triglycerides, median (IQR),mmol/L	1.11 (0.81-1.58)	0.66 (0.57-0.74)	0.88 (0.82-0.94)	1.12 (1.05-1.20)	1.47 (1.35-1.59)	2.23 (1.93-2.78)
Total cancer risk - no. (%)	15,686 (6.1)	2,548 (4.7)	2,746 (5.6)	3,012 (5.9)	3,392 (6.7)	3,988 (7.8)
Follow-up, mean±SD (median), y	11.9±7.2 (10.1)	11.6±7.2 (9.9)	12.1±7.3 (10.3)	11.9±7.2 (10.2)	12.1±7.2 (10.3)	11.8±7.0(10.1)
Person years at risk	3,052,493	634,938	529,501	611,589	611,873	599,995

BMI = body mass index; SD= standard deviation; IQR= interquartile range

Table 2. Relative risk of incident cancer by triglycerides in quintiles and per log-unit increment in men

Site (ICD-7)	n cases	Quintile 1-5. relative risk (95% CI) *						
		1 (Ref)	2	3	4	5	p for trend	Relative risk (95% CI) per log-unit increment of STG*
Total cancer	23.026	1.00	1.14 (1.04-1.24)	1.10 (1.02-1.20)	1.18 (1.10-1.29)	1.16 (1.06-1.26)	0.001	1.10(1.06-1.16)
Lip. oral cavity. pharynx (140-149)	588	1.00	1.12 (0.65-1.91)	1.49 (0.89-2.49)	1.65 (1.00-2.76)	1.51 (0.89-2.58)	0.047	1.49 (1.10-2.04)
Stomach (151)	852	1.00	0.89 (0.58-1.37)	1.12 (0.73-1.68)	1.14 (0.75-1.75)	1.04 (0.67-1.60)	0.49	0.94 (0.73-1.24)
Colon (153)	1.807	1.00	1.44 (1.06-1.96)	1.24 (0.91-1.70)	1.68 (1.24-2.26)	1.96 (1.44-2.67)	<0.001	1.49 (1.24-1.78)
Rectum. Anus (154)	1.154	1.00	1.22 (0.83-1.78)	1.4 (0.96-2.01)	1.37 (0.94-1.98)	1.26 (0.85-1.85)	0.21	1.20 (0.96-1.51)
Liver. intrahepatic bile ducts (155.0)	181	1.00	1.31 (0.50-3.39)	0.5 (0.18-1.44)	1.56 (0.62-3.88)	0.68 (0.25-1.85)	0.61	0.83 (0.46-1.46)
Gallbladder. biliary tract (155.1-155.3)	93	1.00	0.55 (0.14-2.15)	0.36 (0.09-1.51)	1.31 (0.39-4.41)	1.26 (0.36-4.37)	0.28	1.51 (0.70-3.32)
Pancreas (157)	524	1.00	0.94 (0.55-1.63)	1.08 (0.64-1.83)	1 (0.59-1.73)	1.02 (0.58-1.75)	0.90	1.02 (0.73-1.44)
Larynx. trachea/bronchus/lung (161. 162)	2.986	1.00	1.26 (1.02-1.60)	1.14 (0.82-1.44)	1.31 (1.04-1.65)	1.42 (1.12-1.80)	0.007	1.20 (1.04-1.37)
Prostate (177)	6.804	1.00	1.14 (0.98-1.33)	1.06 (0.92-1.24)	1.06 (0.91-1.24)	0.83 (0.72-0.98)	0.014	0.89 (0.82-0.98)
Testis (178)	232	1.00	0.94 (0.44-2.04)	1.93 (0.92-3.96)	1.14 (0.50-2.52)	0.94 (0.40-2.26)	0.81	1.08 (0.65-1.75)
Kidney. renal cell (180.0. 180.9)	685	1.00	0.87 (0.52-1.46)	1.14 (0.70-1.75)	1.58 (0.98-2.52)	1.85 (1.14-3.02)	0.001	1.85 (1.40-2.46)
Bladder (181)	1604	1.00	1.06 (0.77-1.44)	1.26 (0.92-1.73)	1.20 (0.89-1.65)	1.16 (0.85-1.60)	0.24	1.14 (0.94-1.40)
Melanoma of skin (190)	1031	1.00	1.24 (0.83-1.83)	1.44 (0.98-2.12)	1.49 (1.02-2.20)	1.49 (1.00-2.20)	0.03	1.24 (0.98-1.56)

Non-melanoma of skin (191)	785	1.00	1.33 (0.87-2.06)	1.06 (0.68-1.68)	1.24 (0.80-1.96)	1.51 (0.94-2.37)	0.17	1.22 (0.92-1.60)
Brain. nervous tissue (193)	396	1.00	0.45 (0.25-0.83)	0.49 (0.27-0.83)	0.7 (0.39-1.24)	0.89 (0.49-1.60)	0.85	1.14 (0.78-1.65)
Thyroid gland (194)	131	1.00	2.64 (0.83-8.84)	3.49 (1.10-11.14)	2.01 (0.596.90)	3.49 (1.04-11.57)	0.14	1.53 (0.80-2.89)
Lymph/hematopoietic tissue (200-209)	1789	1.00	1.1 (0.83-1.46)	0.98 (0.75-1.31)	0.85 (0.64-1.14)	0.94 (0.70-1.26)	0.26	0.94 (0.75-1.14)
Non-Hodgkin's lymphoma (200. 202)	818	1.00	1.02 (0.65-1.49)	1.02 (0.67-1.53)	0.87 (0.56-1.31)	0.61 (0.39-0.96)	0.03	0.7 (0.53-0.92)
Multiple myeloma (203)	320	1.00	1.35 (0.68-2.64)	0.89 (0.44-1.80)	1.10 (0.55-2.17)	1.29 (0.64-2.61)	0.73	1.14 (0.73-1.75)
Leukemia (204-207)	494	1.00	0.87 (0.49-1.51)	0.89 (0.52-1.56)	0.75 (0.43-1.31)	1.44 (0.83-1.02)	0.29	1.53 (1.08-2.15)
Other cancer§	1.102	1.00	1.18 (0.82-1.70)	0.82 (0.55-1.18)	1.04 (0.72-1.51)	1.26 (0.87-1.83)	0.43	1.2 (0.94-1.51)

*Relative risks (RR) were estimated from Cox models with age as time scale, stratified by cohort and birth year, and adjusted for baseline age, and BMI and smoking status, and RRs per 1 log unit increment were additionally adjusted for fasting time. RRs are corrected for random error by regression dilution ratio (RDR); conversion into uncorrected RR = exp(log(RR)*RDR). RDR=0.522

§Other cancer than separately presented sites.

Table 3. Relative risk of incident cancer by triglycerides in quintiles and per log-unit increment in women

Site (ICD-7)	*n* cases	Quintile 1-5, relative risk (95% CI)*						
		1 (Ref)	2	3	4	5	p for trend	Relative risk (95% CI) per log-unit increment
Total cancer	15,658	1.00	1.04 (0.93-1.13)	0.96 (0.88-1.07)	1.04 (0.95-2.80)	1.15 (1.05-1.27)	0.003	1.11 (1.04-1.19)
Lip, oral cavity, pharynx (140-149)	195	1.00	0.73 (0.27-2.00)	1.63 (0.65-4.01)	1.98 (0.81-4.81)	1.79 (0.73-4.45)	0.04	2.18 (1.25-3.74)
Stomach (151)	409	1.00	0.96 (0.49-1.88)	1.21 (0.64-2.28)	1.02 (0.53-1.93)	1.49 (0.81-2.80)	0.16	1.33 (0.89-1.95)
Colon (153)	1,364	1.00	0.81 (0.57-1.15)	0.86 (0.61-1.23)	0.98 (0.70-1.37)	1.05 (0.75-1.47)	0.13	1.05 (0.86-1.31)
Rectum, Anus (154)	659	1.00	0.98 (0.61-1.60)	0.86 (0.53-1.39)	1.00 (0.62-1.60)	1.33 (0.84-2.13)	0.19	1.43 (1.05-1.95)
Liver, intrahepatic bile ducts (155.0)	69	1.00	0.27 (0.05-1.63)	0.43 (0.09-2.10)	0.78 (0.18-3.33)	1.29 (0.32-5.14)	0.23	2.23 (0.89-5.48)
Gallbladder, biliary tract (155.1-155.3)	97	1.00	2.68 (0.58-1.04)	3.39 (0.78-14.6)	2.10 (0.47-9.41)	1.83 (0.41-7.77)	0.94	1.17 (0.54-2.60)
Pancreas (157)	321	1.00	0.70 (0.33-1.47)	0.86 (0.42-1.72)	0.86 (0.43-1.72)	1.17 (0.60-2.25)	0.38	1.31 (0.84-2.03)
Larynx, trachea/bronchus/lung (161, 162)	847	1.00	1.11 (0.73-1.72)	1.05 (0.68-1.60)	2.00 (1.35-2.97)	2.10 (1.41-3.12)	<0.001	1.69 (1.43-2.31)
Breast (170)	5,006	1.00	1.05 (0.89-1.25)	0.88 (0.75-1.04)	0.91 (0.78-1.07)	0.95 (0.79-1.11)	0.2	0.91 (0.81-1.02)
Cervix (171)	434	1.00	1.13 (0.64-2.00)	1.23 (0.70-2.15)	1.05 (0.60-1.88)	1.88 (1.07-3.30)	0.05	1.65 (1.15-2.41)
Other parts of uterus (172, 174)	1,149	1.00	1.27 (0.88-1.83)	0.95 (0.65-1.37)	1.04 (0.71-1.49)	1.23 (0.86-1.76)	0.52	1.13 (0.89-1.41)
Ovary (175.0)	753	1.00	1.09 (0.70-1.69)	1.31 (0.84-1.98)	1.11 (0.73-1.72)	1.05 (0.68-1.65)	0.20	0.95 (0.71-1.27)

Kidney, renal cell (180.0, 180.9)	319	1.00	0.79 (0.40-1.60)	0.47 (0.23-0.96)	0.84 (0.43-1.63)	0.79 (0.41-1.54)	0.78	0.98 (0.64-1.52)
Bladder (181)	341	1.00	0.51 (0.25-1.04)	0.75 (0.40-1.43)	0.64 (0.34-1.23)	0.88 (0.46-1.65)	0.87	1.02 (0.67-1.58)
Melanoma of skin (190)	691	1.00	0.98 (0.65-1.49)	0.95 (0.61-1.43)	0.84 (0.54-1.31)	0.73 (0.46-1.15)	0.14	0.78 (0.57-1.05)
Non-melanoma of skin (191)	397	1.00	1.58 (0.79-3.12)	1.00 (0.50-2.03)	1.29 (0.65-2.52)	1.98 (1.04-3.84)	0.07	1.37 (0.93-2.03)
Brain, nervous tissue (193)	258	1.00	0.47 (0.22-1.05)	0.93 (0.46-1.86)	0.79 (0.39-1.63)	1.39 (0.70-2.83)	0.16	1.43 (0.88-2.31)
Thyroid gland (194)	243	1.00	1.65 (0.76-3.52)	1.74 (0.81-3.71)	1.52 (0.70-3.30)	1.07 (0.47-2.44)	0.97	0.98 (0.60-1.63)
Lymph/hematopoietic tissue (200-209)	1,077	1.00	1.19 (0.81-1.72)	1.21 (0.84-1.74)	0.89 (0.62-1.31)	1.00 (0.68-1.45)	0.45	1.00 (0.79-1.29)
Non-Hodgkin's lymphoma (200, 202)	539	1.00	1.27 (0.75-2.15)	1.49 (0.89-2.49)	1.09 (0.64-1.83)	0.84 (0.49-1.45)	0.29	0.91 (0.65-1.29)
Multiple myeloma (203)	212	1.00	1.13 (0.51-2.57)	0.79 (0.35-1.81)	0.64 (0.28-1.47)	0.81 (0.36-1.86)	0.32	1.07 (0.62-1.83)
Leukemia (204-207)	251	1.00	1.11 (0.51-2.44)	0.98 (0.46-2.15)	0.65 (0.30-1.45)	1.19 (0.55-2.55)	1.00	1.02 (0.62-1.67)
Other cancer§	778	1.00	1.15 (0.71-1.83)	1.25 (0.79-1.98)	1.63 (1.04-2.52)	1.63 (1.04-2.55)	0.009	1.43 (1.09-1.90)

*Relative risks (RR) were estimated from Cox models with age as time scale, stratified by cohort and birth year, and adjusted for baseline age, and BMI and smoking status, and RRs per 1 log unit increment were additionally adjusted for fasting time. RRs are corrected for random error by regression dilution ratio (RDR); conversion into uncorrected RR = exp(log(RR)*RDR). RDR=0.555

§Other cancer than separately presented sites.

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Serum triglyceride concentrations and cancer risk in a large cohort study in Austria

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BACKGROUND: Blood lipid levels as part of the metabolic syndrome are thought to be linked to cancer risk. Few epidemiological studies have addressed the association between serum triglyceride (STG) concentrations and cancer risk.

METHODS: Serum triglyceride concentrations were collected in a health investigation (1988–2003). The analyses included 156 153 subjects (71 693 men and 84 460 women), with 5079 incident cancers in men and 4738 cancers in women, and an average of 10.6 years of follow-up. All malignancies were ascertained from the population cancer registry. Multivariate Cox proportional hazard models stratified by age and sex were used to determine adjusted cancer risk estimates and 95% confidence interval (95% CI).

RESULTS: In men and women combined, higher STG concentrations were associated with increased risk of lung (4th *vs* 1st quartile: HR, 1.94; 95% CI, 1.47–2.54), rectal (HR, 1.56; 95% CI, 1.00–2.44), and thyroid cancer (HR, 1.96; 95% CI, 1.00–3.84). Serum triglyceride concentrations were inversely associated with non-Hodgkin's lymphoma. In men, STG concentrations were inversely associated with prostate cancer and positively with renal cancer. In women, STG concentrations were positively associated with gynaecological cancers. Stratification by BMI revealed a higher risk of gynaecological cancers in overweight than in normal weight women. No other associations were found.

CONCLUSIONS: Our findings support the hypothesis that STG concentrations are involved in the pathogenesis of lung, rectal, thyroid, prostate, and gynaecological cancers.

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Obesity has been identified as a major risk factor for such cancer sites as colon, renal, breast, and endometrium (Bianchini *et al*, 2002; Calle and Kaaks, 2004; Rapp *et al*, 2005), whereas hypertriglyceridemia is relevant to obesity and insulin resistance (Despres and Lemieux, 2006). Dietary fat intake is a well-established risk factor in cardiovascular diseases (CVDs), in which much investigation has involved serum triglyceride (STG) concentrations (Sarwar *et al*, 2007). The combination of hypertriglyceridemia and elevated waist circumference has been identified as a phenotype for higher risk of CVD (Kahn and Valdez, 2003). Usually, fasting triglyceride concentrations are measured, as they are associated with increased mortality and CVD risk (Brunzell, 2007). However, there is uncertainty with regard to the impact of STG concentrations on risk of CVD (Gotto, 1998 and also with regard to whether fasting level influences the relationship (Langsted *et al*, 2008). Beyond lipid metabolism there is evidence that hypertriglyceridemia is associated with frequent infections and inflammation (Khovidhunkit *et al*, 2004; Esteve *et al*, 2005).

A few cohort studies have investigated high STG concentrations as a part of the metabolic syndrome (Tulinius *et al*, 1997) in relation to risk of colon (Saydah *et al*, 2003; Ahmed *et al*, 2006, Tande *et al*, 2006), breast (Vatten and Foss, 1990; Furberg *et al*, 2004), and cervix cancers (Cust *et al*, 2007). A cohort study among Icelanders (Tulinius *et al*, 1997) revealed associations between high STG levels and colorectal cancers in both sexes, and also with thyroid cancer in men, as well as with cervix, endometrial, and bladder cancer in women (Tulinius *et al*, 1997).

We therefore investigated the associations between fasting STG concentrations and cancer risk in a large prospective cohort study.

METHODS

Study population

Details of the Vorarlberg Health Monitoring and Promotion Program (VHM&PP) in Vorarlberg, the most western region in Austria, are provided elsewhere (Rapp *et al*, 2005). In brief, the Agency of Social and Preventive Medicine annually offered to all adults living in Vorarlberg a screening examination that includes a physical examination, a blood test, and a consultation with a doctor. By 2005, ~56% of all Vorarlberg residents underwent at least one examination in this voluntary screening programme. Between 1988 and 2003, over 156 000 adult Vorarlberg residents were enrolled in the cohort after signing an informed consent form to store and process personal data and biological samples.

For the current analysis, we used a data set with complete data on STG and covariates at baseline. Participants with follow-up

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Table 1 Characteristics of the study population ($N = 156\,153$) by quartile of serum triglyceride (STG) concentration

	All	1st quartile	2nd quartile	3rd quartile	4th quartile
Triglycerides (mg 100 ml^{-1}), median (Q1, Q3)	104.0 (74.0, 152.0)	61.0 (52.0, 68.0)	88.0 (81.0, 96.0)	124.0 (114.0, 137.0)	207.0 (175.0, 269.0)
Total cholesterol (mg 100 ml^{-1}), mean (s.d.)	213.7 (45.8)	188.8 (36.3)	206.1 (39.2)	220.0 (41.7)	240.9 (48.5)
Glucose (mg 100 ml^{-1}), mean (s.d.)	87.2 (22.6)	83.7 (16.3)	85.0 (20.0)	87.2 (21.0)	93.0 (31.2)
Gamma-glutamyltransferase (GGT) (U l^{-1}), median (Q1, Q3)	12.0 (8.0, 18.0)	9.0 (7.0, 13.0)	11.0 (8.0, 15.0)	12.0 (9.0, 19.0)	18.0 (12.0, 29.0)
Age (years), mean (s.d.)	41.8 (15.1)	37.2 (13.0)	40.8 (15.1)	43.4 (15.9)	50.0 (14.9)
BMI (kg m^{-2}), mean (s.d.)	24.7 (4.2)	22.9 (3.4)	24.1 (3.9)	25.2 (4.3)	30.0 (4.3)
Current smokers (%)	25.2	20.4	23.7	26.1	30.8
Occupational status: white collar (%)	54.3	58.3	56.1	53.3	49.3

<1 year ($n = 6188$), or with prevalent cancer (other than non-melanoma skin cancer), were excluded before enrolment or within 1 year after enrolment ($n = 2149$).

Two central laboratories, with regular internal and external quality tests, determined STG concentrations on fasting blood samples. Within 60–240 min of venous blood sample collection from a cubital vein, serum was obtained by centrifugation for 15 min at 4000 r.p.m. Subsequently, STG concentrations were measured at 37°C and were expressed as mg per 100 ml. To check calibration, three daily control samples were included. If average values of control samples of each run were not within 3% of the true value, the run was repeated. Day-by-day variation had to be within 5%. Study participants are classified according to the quartiles of STG concentrations with the following cutoff values: ⩽83, 84–119, 120–179, and ⩾180 mg 100 ml for men and ⩽69, 70–94, 95–133, and ⩾134 mg 100 ml for women. Participants in the 1st quartile were used as reference category.

Measurements of height, weight, blood pressure, total cholesterol, blood glucose, and gamma-glutamyltransferase (GGT) were obtained routinely for each participant. BMI was calculated by height and weight at baseline and was categorised on the basis of clinical guidelines (<25 kg m^{-2}, 25 to <30 kg m^{-2}, ⩾30.0 kg m^{-2}) (World Health Organisation, 1998). Smoking status was classified as current, former, or non-smokers. Participants who never smoked could not be distinguished from those who did not respond to questions with regard to smoking at baseline, but baseline smoking status was verified for >70% of study participants on the basis of information provided at subsequent examinations. As a proxy for socio-economic position, the occupational group (blue collar, white collar, or self-employed) was determined by the participant's insurance number. Retired participants were classified according to their former occupation, and housewives on the basis of the job of their spouse.

As described previously in detail (Rapp *et al*, 2005), cancer cases were identified by record linkage with the Vorarlberg cancer registry, which has been accepted for IARC publication since 1993 (Parkin DM *et al*, 2003) and has high completeness of ascertainment (Oberaigner W, 2006). In the Vorarlberg cancer registry, nearly all cancers (96.7%) were histologically verified and the Death-certificate-only (DCO) rate meets international quality criteria (5% for both sexes in 1998–2002). Cohort data were linked to the Vorarlberg Death Index to identify deaths and to calculate person-years. The current analysis makes use of the data set updated at the end of 2003. The average follow-up time was 10.6 (s.d. 4.5) years. The 10th Revision of the *International Statistical Classification of Diseases, Injuries and Causes of Death* (ICD) was used to code the cancers (World Health Organization, 2008).

Statistical analysis

The analytical cohort comprised 156 153 subjects (71 693 men and 84 460 women). Partial correlation coefficients were calculated to examine the relationship between STG and other clinical parameters. Cox proportional hazard models were used to compute hazard ratios (HRs) and 95% confidence intervals (CIs) for quartiles relative to the reference group (1st quartile of STG level). Models were adjusted for serum concentrations of glucose (mg per 100 ml, continuous)(Rapp *et al*, 2006), total cholesterol (mg per 100 ml, continuous) (Ulmer *et al*, 2004; Strasak *et al*, 2009), GGT (U l^{-1}, continuous) (Strasak *et al*, 2008a,b), body mass index (BMI, kg m^{-2}, continuous) (Rapp *et al*, 2005), occupational status, and smoking status (both in classes). Continuous risk estimates are presented for an increase in exposures of one unit log-transformed STG concentration. To test the overall significance of exposure, *P*-values for Wald χ^2 statistics are shown. As no obvious sex differences between the estimates emerged, models were calculated for the sexes combined. All *P*-values are two-sided and all calculations were carried out with SAS statistical software package SAS release 9.1 (SAS Institute, Cary, NC, USA).

Table 2 Correlation of serum triglyceride (STG) concentrations with other clinical measures in the study population

Covariates	Correlation coefficients[a]	*P*-value
BMI (kg m^{-2})	0.30	<0.001
Total cholesterol (mg 100 ml^{-1})	0.41	<0.001
Glucose (mg 100 ml^{-1})	0.14	<0.001
Gamma-glutamyltransferase (GGT) (U l^{-1})	0.32	<0.001

[a]Age- and sex- adjusted partial correlation coefficients, STG, and GGT were log transformed.

RESULTS

During follow-up, 5079 incident invasive cancer cases among men and 4738 incident invasive cancer cases among women were identified (Table 1). Correlations between BMI, age, and serum concentrations of STG, total cholesterol, glucose, and GGT are shown in Table 2. STG was weakly associated with serum glucose concentrations and moderately associated with BMI, total cholesterol, and GGT concentrations.

Table 3 shows the hazard ratios for cancer type by STG concentrations in the VHM&PP cohorts. Compared with the 1st quartile, high STG concentrations (4th quartile) were associated with increased risk of lung (HR, 1.94; 95% CI, 1.47–2.54), rectal (HR, 1.56; 95% CI, 1.00–2.44), and thyroid cancer (HR, 1.96; 95% CI, 1.00–3.84). High STG concentrations were inversely associated with non-Hodgkin's lymphoma. Prostate cancer was inversely associated with STG concentrations (per log-unit HR, 0.80; 95% CI, 0.72–0.90) and was positively associated with incidence of kidney cancer in men (data not shown). High STG concentrations were associated with higher overall cancer risk (4th *vs* 1st quartile: HR, 1.19; 95% CI, 1.05–1.33) and with risk of gynaecological cancers (endometrium, ovar, cervix) (4th *vs* 1st quartile: HR, 1.62; 95% CI, 1.13–2.33).

Table 3 Hazard ratios (HR with 95% CI) and numbers of cases for cancers by site and STG quartiles[a]

Cancer site of both sexes ICD-10 codes	Total no. of cases	1st quartile	2nd quartile	3rd quartile	4th quartile	Total per log-unit increase	*P*-value for log unit increase
Thyroid C73	(101)	(16) 1.00	(25) 1.55 (0.82–3.00)	(30) 1.84 (0.97–3.48)	(30) 1.96 (1.00–3.84)	1.16 (0.76–1.76)	0.492
Plasmacytoma C90	(73)	(7) 1.00	(19) 1.88 (0.74–4.78)	(16) 1.37 (0.52–3.59)	(31) 2.11 (0.82–5.43)	1.39 (0.84–2.31)	0.204
NHL C82–C85	(219)	(42) 1.00	(61) 0.99 (0.66–1.49)	(54) 0.71 (0.46–1.07)	(62) 0.68 (0.43–1.07)	0.71 (0.52–0.97)	0.033
Stomach C16	(315)	(41)	(80) 1.32 (0.90–1.95)	(83) 1.22 (0.83–1.82)	(111) 1.45 (0.97–2.17)	1.17 (0.92–1.49)	0.208
Pancreatic C25	(162)	(24) 1.00	(35) 0.99 (0.58–1.69)	(39) 0.86 (0.50–1.47)	(64) 1.19 (0.70–2.05)	1.25 (0.90–1.75)	0.188
Colon C18	(600)	(81) 1.00	(122) 0.93 (0.70–1.23)	(175) 1.05 (0.80–1.39)	(222) 1.08 (0.81–1.43)	1.06 (0.88–1.26)	0.547
Rectal C19/20	(273)	(32) 1.00	(69) 1.47 (0.95–2.26)	(69) 1.25 (0.81–1.94	(103) 1.56 (1.00–2.44)	1.20 (0.92–1.55)	0.184
Bladder C67	(158)	(24) 1.00	(36) 1.50 (0.48–4.71)	(59) 0.73 (0.22–2.46)	(39) 1.42 (0.45–4.43)	1.03 (0.98–1.09)	0.218
Kidney C64	(216)	(35) 1.00	(40) 0.81 90.51–1.280	(51) 0.88 (0.57–1.38)	(90) 1.27 (0.81–1.97)	1.27 (0.95–1.69)	0.105
Lung C34	(650)	(86) 1.00	(128) (1.12 0.85–1.48)	(179) (1.43 1.09–1.87)	(257) (1.94 1.47–2.54)	1.50 (1.28–1.75)	<0.0001
Men							
Prostate C61	(1484)	(304) 1.00	(397) 0.94 (0.80–1.09)	(470) 0.87 (0.75–1.02)	(353) 0.67 (0.56–0.80)	0.80 (0.72–0.90)	<0.001
Women							
Breast C50 ⩽50 years	(510)	(127) 1.00	(157) 1.25 (0.98–1.59)	(136) 1.23 (0.95–1.59)	(90) 0.95 (0.70–1.28)	0.92 (0.74–1.15)	0.455
Breast >50 years	(694)	(75) 1.00	(135) 0.93 (0.70- 1.24)	200 1.00 (0.76–1.32)	284 1.05 (0.79–1.39)	1.09 (0.91–1.30)	0.352
Cervical C53	(70)	(12) 1.00	(17) 1.48 (0.69–3.19)	(18) 1.52 (0.70–3.34)	(23) 2.00 (0.89- 4.50)	1.74 (1.03–2.95	0.038
Endometrium C54	(236)	(22) 1.00	(50) 1.38 (0.83–2.30)	(54) 1.11 (0.66–1.86)	(110) 1.61 (0.97–2.67)	1.22 (0.90–1.65)	0.206
Ovarian C56	(123)	(16)	(29) 1.57 (0.81–3.04)	(39) 1.75 (0.91–3.37)	(39) 1.43 (0.71–2.85)	1.13 (0.74–1.74)	0.576
Gynaecological (C53, 54, 56)	(429)	(50) 1.00	(96) 1.45 (1.01- 2.07)	(111) 1.35 (0.94–1.93)	(172) 1.62 (1.13–2.33)	1.26 (1.01–1.58)	0.042

[a]Adjusted for BMI (kg m^{-2}, continuous), GGT (continuous), serum glucose (continuous), total cholesterol concentration (continuous), smoking status, and occupational status.

Figure 1 shows HRs for selected cancers by STG concentrations stratified by BMI. These did not reveal differential associations of STG levels with cancer overall, or with lung and colon cancer risk; however, the risk of gynaecological cancers was higher in overweight than in normal weight women. When data were stratified by smoking status (data not shown), no differential estimates emerged for overall and gynaecological cancers, but a somewhat higher risk of rectal cancer was found in current smokers ($N=50$ cases; per log-unit HR, 1.73; 95% CI, 1.02–2.92) than in non-smokers ($N=177$ cases; per log-unit HR, 1.11; 95% CI, 0.79–1.56), and a higher risk of lung cancer was found in non-smokers ($N=222$ cases; per log-unit HR, 1.57; 95% CI, 1.19–2.06) than in smokers ($N=334$ cases; per log-unit HR, 1.13; 95% CI, 0.91–1.39). However, no significant effect modification by BMI and smoking status was found. Stratification by GGT levels ($\leqslant 30$ and >30 U l^{-1}) revealed differential relationships between STG and overall cancer risk and lung cancer (data not shown).

DISCUSSION

In this large-scale cohort study, high STG concentrations were associated with higher overall cancer risk in women, but not in men. In men and women combined, STG concentrations were related to high risk of lung, thyroid, and rectal cancer. In men, STG concentrations were associated inversely with prostate cancer, and in women they were associated positively with gynaecological cancers. Our findings regarding lung, rectal, and gynaecological cancers are consistent with data using dietary fat intake levels as exposure variable (Kushi and Giovannucci, 2002; Genkinger *et al*, 2006).

Our observation of a positive association between STG levels and rectal cancer is in line with previous findings in a cohort study among Icelanders (Tulinius *et al*, 1997). Further evidence for a relationship with STG comes from case–control studies on colorectal adenoma (Kono *et al*, 1990; Bird *et al*, 1996; Otani *et al*, 2006; Tabuchi *et al*, 2006), carcinoma *in situ* (Yamada *et al*, 1998), and from an *in-vitro* study (Tabuchi *et al*, 2008). It has been suggested that total cholesterol, STG, and plasma glucose are positively associated with colorectal cancer risk (Yamada *et al*, 1998). In our study, the association occurred adjusted for plasma glucose and total cholesterol concentrations. In men and women combined, we observed an association between STG concentration and rectal cancer risk, whereas no association was found for colon cancer, neither was any association found in sex-stratified analyses. Consistent with a study among Japanese-American men (Tsushima *et al*, 2005) and US prospective studies (Saydah *et al*, 2003; Ahmed *et al*, 2006), we did not find a relationship with colorectal cancer.

Our findings of high lung cancer risk among subjects with high STG concentrations are unique. In one study, an association between total cholesterol and lung cancer risk has been observed, suggesting a relationship between lipid metabolism and lung cancer risk (Hinds *et al*, 1983). In the Carotene and Retinol Efficacy Trial (CARET), among the participants receiving β-carotene and retinol, higher serum triglyceride concentrations were observed (Cartmel *et al*, 2005), suggesting a relationship between STG and lung cancer risk. As smoking is associated with higher STG concentrations (Brunzell, 2007), residual confounding due to smoking may contribute to the association between STG concentrations and lung cancer risk. In our study, however, the association persisted when the data set was limited to non-smokers, suggesting that factors other than smoking status may contribute to the observed association. The limited differentiation between missing smoking data and non-smoking status may have resulted in misclassification of smoking status. However, smoking information from follow-up visits for most of the participants was used to complement the baseline smoking status.

The positive association with thyroid cancer risk is in line with findings in a cohort study (Tulinius *et al*, 1997). It may be relevant that BMI was positively associated with thyroid cancer (Renehan *et al*, 2008).

In our study, STG concentrations were inversely associated with prostate cancer risk, in contrast to the reverse findings in a case–control study (Wuermli *et al*, 2005). However, in this clinical-based study, prostate cancers were compared with benign prostate hyperplasia, in which STG levels were lower than those in cancer cases. In large cohort studies in Norway and the United States, no association between STG and prostate cancer risk was found (Lund

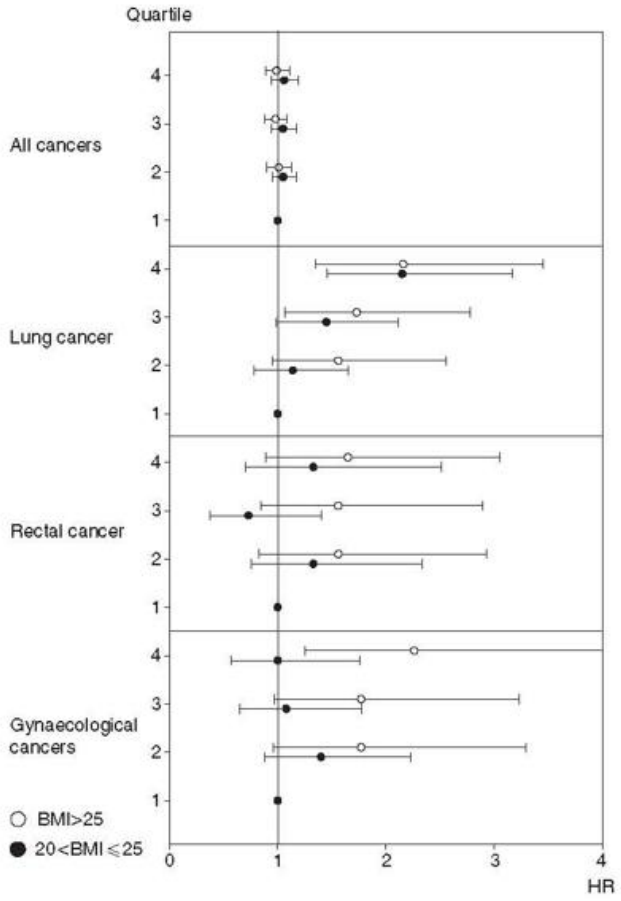

Figure 1 Incidence of selected cancer sites according to sex-specific quartiles of serum triglyceride concentrations in the study population ($N = 156\,153$) by BMI*. *Adjusted for BMI (kg m^{-2}, continuous), GGT (continuous), serum glucose (continuous), total cholesterol concentration (continuous), smoking status, occupational status, and sex (not for gynaecological cancers).

et al, 2006; Tande *et al*, 2006). The application of prostate-specific antigen contributes to heterogeneity of phenotype (Etzioni *et al*, 2002), which may have distorted the relationship with STG. In addition, an inverse relationship for NHL was observed in our study. Previous reports on cholesterol indicted that reverse causation may substantially contribute to the risk-lowering effect of high blood lipids (Rose and Shipley, 1980; Lim *et al*, 2007; Strasak *et al*, 2009).

Our observation that STG concentrations were positively associated with kidney cancer incidence in men (data not shown) contrasts with a recently published meta-analysis on BMI and renal cancer risk (Renehan *et al*, 2008). However, in our study, after adjusting for diastolic blood pressure, an established risk factor for renal cancer (per log-unit HR, 1.26; 95% CI, 0.95–1.68), the association was no longer statistically significant. For men and women combined, we found no statistically significant association between STG and kidney cancer risk.

Our observation of a positive association of STG concentrations with risk of gynaecological cancers (cervix, ovary, endometrial) is consistent with other studies (Tulinius *et al*, 1997; Cust *et al*, 2007). In one study, increasing triglyceride and glucose concentrations were associated with increased endometrial cancer risk (Cust *et al*, 2007). Our findings on cervical cancer are in line with those in a cohort study (Tulinius *et al*, 1997). For breast cancer, inconsistent results have been reported from a nested case–control study (Agnoli *et al*, 2009) and from cohort studies (Vatten and Foss, 1990; Furberg *et al*, 2004).

These associations with gynaecological cancer raise a question with regard to the involvement of oestrogens, which are considered to stimulate hepatic triglyceride secretion (Sattler *et al*, 2005), as confirmed by studies on hormone replacement therapy (Rossouw *et al*, 2008; Sowers *et al*, 2008).

High STG concentrations may reflect other metabolic aspects that are procarcinogenic (McKeown-Eyssen, 1994). Associations between STG and plasma glucose levels are well established and hyperglycaemia is a risk factor for several cancers (Ashley and Kannel, 1974). In our study, however, we used fasting STG levels and adjusted for plasma glucose levels to control for confounding by glucose levels. Inflammation is another potential mechanism by which hypertriglyceridemia is associated with cancer risk (Esteve *et al*, 2005; Kundu and Surh, 2008). STG concentrations may be linked to colorectal cancer risk by bile acid excretion, circulation hormones, or energy supply to neoplastic cells (McKeown-Eyssen, 1994).

A limitation of our study is the lack of information on such potential risk factors as alcohol consumption and physical activity. However, the results of our multivariate models adjusted for GGT concentrations may be considered as a proxy variable for alcohol intake (Whitehead *et al*, 1978). In addition, no information on medication history (for example, on lipid-lowering drugs or hormones) was available, which may have affected the associations observed. Among women, the effect of STG may be overestimated because of residual confounding by exogenous hormones, whereas for lipid-lowering medications, the opposite could be relevant.

Undocumented measurement variation in STG concentrations during the study period may also have affected our results, but we assume that these were minor, as we used fasting STG levels. The strengths of our study are large sample size, prospective design, length of follow-up, and standardised examinations by trained physicians. It is relevant that the study population is relatively young and healthy.

Overall, STG concentrations were positively associated with the risk of lung, thyroid, and rectal cancers, but inversely with NHL risk. Prostate cancer risk was inversely associated with STG concentrations, whereas positive associations were found with renal cancer among men and with gynaecological cancers among women. Our results suggest that STG concentrations are involved in the pathogenesis of several cancer sites.

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Epidemiology

Metabolic risk factors and primary liver cancer in a prospective study of 578,700 adults

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Short title:

Metabolic risk factors and primary liver cancer

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Abbreviations:

Me-Can	Metabolic syndrome and Cancer project
RR	Relative risk
BMI	Body mass index
SD	Standard deviation
Mets	Metabolic syndrome
HCC	Hepatocellular carcinoma
PLC	Primary liver cancer
ICC	Intrahepatic cholangiocarcinoma
HCV	hepatitis C virus
NCS	Norwegian Counties Study
CONOR	Cohort of Norway
40-y	Age 40-programme
VIP	Västerbotten Intervention Project
VHM&PP	Vorarlberg Health Monitoring and Prevention Programme
Oslo	Oslo study I
MPP	Malmö Preventive Project
mmol/L	millimol per liter
ICD	International Classification of Diseases
DCO	Death certificate only
Mm_ MAST	Malmo modification of the brief Michigan Alcoholism Screening Test
RDR	Regression dilution ratio
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis

Abstract

Initial studies have indicated diabetes and obesity to be risk factors for hepatocellular carcinoma; but the association between other metabolic risk factors and primary liver cancer has not been investigated.

The metabolic syndrome and cancer project (Me-Can) includes cohorts from Norway, Austria, and Sweden with data on 578,700 subjects. We used Cox proportional hazard models to calculate relative risks (RRs) of primary liver cancer by body mass index (BMI), blood pressure, and plasma levels of glucose, cholesterol, and triglycerides as continuous standardized variables (z-score with mean=0 and standard deviation (SD)=1) and their standardized sum of metabolic syndrome (Mets) z-score. RRs were corrected for random error in measurements.

During an average follow-up of 12.0 years (SD=7.8), 266 primary liver cancers were diagnosed among cohort members. Relative risk of liver cancer per unit increment of z-score adjusted for age, smoking status and BMI and stratified by birth year, sex and sub-cohorts, was for BMI 1.39 (95% confidence interval (CI) 1.24 to 1.58), blood glucose 2.13, (1.55 to 2.94) and for cholesterol. 0.62, (0.51 to 0.76). The RR per one unit increment of the MetS z-score was 1.35, (1.12 to 1.61).

BMI, glucose, and a composite metabolic syndrome score were positively and cholesterol negatively associated with risk of liver cancer.

Introduction

Primary liver cancer is the sixth most common cancer in the world and is characterised by high mortality. It is estimated to cause over half a million deaths per year world-wide [1]. Owing to its high fatality, the incidence and mortality rates are almost equal with the mortality to incidence ratio being almost a unity [2]. Hepatocellular carcinoma (HCC) is by far the major histological subtype and accounts for up to 85% of PLCs [3]. Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer, which accounts for up to 20% of the PLC [3]. Although both histological subtypes are characterized by high mortality, there are differences in the risk factors for their development [1-3].

Chronic hepatitis B and C viral infections are major risk factors for hepatocellular carcinoma contributing to the high incidence of this cancer in Asia and Sub-Saharan Africa. In European populations, however, the prevalences of hepatitis B and C are low, <7% (even <2% in North and central Europe) and <2% respectively [3, 6, 7]. Another well-established risk factor is heavy and prolonged alcohol consumption which leads to alcoholic cirrhosis and eventually to HCC [3]. In contrast to hepatocellular carcinoma, the etiology and pathogenesis of cholangiocarcinomas remain poorly understood. Most of these cancers develop in an otherwise normal liver and only about 10% of cases are preceded by chronic inflammatory disease processes such as primary sclerosing cholangitis, hepatic fluke infestations and hepatholithiasis [5].

The incidence and mortality of primary liver cancer is increasing and more strikingly so in Western countries [7, 8-12]. Approximately half of the increase is attributable to hepatitis C virus infection [9], but other major contributors to the rise in disease burden remain to be determined. The parallel increase of the incidence of PLC and that of obesity and type 2-diabetes, shown to be directly associated with risk in some studies, indicate that metabolic alterations may play a role [13-15].

The aim of this prospective study was to investigate the association between metabolic risk factor components, namely BMI, blood pressure, glucose, cholesterol and triglycerides (individually and combined), and primary liver cancer risk.

Materials and methods

Study population and measurements

The Metabolic syndrome and Cancer project (Me-Can) includes cohorts with 578,700 participants from Norway (the Oslo study I cohort-Oslo, the Norwegian Counties Study-NCS, the Cohort of Norway-CONOR, and the Age 40-programme-40-y), Austria (the Vorarlberg Health Monitoring and Prevention Programme-VHMPP), and Sweden (the Västerbotten Intervention Project-VIP, and the Malmö Preventive Project-MPP). A detailed description of Me-Can, and inclusion criteria for participants in this study, has been previously described [16]. In these cohorts, health examinations were performed in 1972 or later, from which data are available on height, weight, blood pressure, blood levels of glucose, total cholesterol, triglycerides, smoking status and alcohol consumption (only for MPP). The study project was approved by research ethical committees in Norway, Austria, and Sweden.

Follow-up and endpoints

In all three countries, incident and fatal cases of primary liver cancer (International Classification of Diseases, seventh revision (ICD-7): 155.0) were identified through linkages with national cancer registries and the respective National Cause of Death Register, and in Norway and Sweden, data on emigration were available through linkages to the Registers of the Total Population and Population Changes.

In our data, PLC was defined as hepatocellular carcinoma (HCC) or intrahepatic cholangiocarcinoma (ICC). Adenocarcinomas were considered ineligible for the study as the vast majority of them are metastatic lesions. PLC events also included death certificate cases only (DCO) that are events where the only source of information about the case was a death certificate. To reduce the possible role of reverse causation, follow-up started one year after the baseline examination. Follow-up ended at the date of the first cancer diagnosis, emigration, death or December 31, 2003 (Austria), 2005 (Norway) and 2006 (Sweden).

Statistical analysis

Cox proportional hazards regression models with age as the time variable, were fitted to obtain hazard ratios, denoted as relative risks (RRs), for PLC events with 95% confidence intervals (95% CI). Quintile cut-off points for the exposure variables were cohort and sex-specific and for glucose, cholesterol and triglycerides, cut-offs were also specific for fasting time before blood sampling (>8 hours, fasting or ≤8 hours, non-fasting). The models were stratified for seven sub-cohorts, sex and year of birth (five categories; ≤1929, 1930-39, 1940-49, 1950-59 and ≥1960), and adjusted for age, smoking status (three categories; never, former and current smokers) and further adjusted for BMI in a second model. To test for trend across quintiles we used mean levels within sub-cohorts and fasting time specific quintiles of the exposure variables.

Further analysis was undertaken with the variables on a standardized continuous scale. All exposure variables were standardized to z-score variables with mean=0 and standard deviation (SD)=1. A composite blood pressure variable was computed as the mean of the systolic and diastolic measurements. The variables were standardized separately across sub-cohorts and fasting time as in the quintile classification. As glucose and triglycerides were skewed and had outliers, they were logarithmically transformed prior to standardization. A score for the metabolic syndrome, constructed by adding the individual z-scores, were also standardized to a z-score variable with mean=0 and SD=1, with the same stratification as above. As in the quintile analyses, the models were stratified for seven sub-cohorts, sex and year of birth (five categories; ≤1929, 1930-39, 1940-49, 1950-59 and ≥1960), and adjusted for age at measurement, smoking status (three categories; never, former and current smokers) and in a further model also for BMI. In one of the Swedish sub-cohorts (MPP) a sub analysis was done with further adjustment for alcohol consumption. Alcohol consumption was assessed by a scoring system based on a modified version of the Michigan Alcoholism Screening Test, previously referred to as the 'Malmö modification of the brief Michigan Alcoholism Screening Test' (Mm-MAST) [17-18]). Based on this scoring system, individuals were classified into "low", "intermediate" and "high" risk.

All risk estimates were adjusted for random error in exposure measurements and within-person variability by use of methods based on regression dilution ratio (RDR). The calculations are based on data from 133 820 participants who have undergone repeated measurements in Me-Can and for whom two or more observations with the same fasting time before measurements were available, in total 406 364 observations. The mean time between the baseline

measurement and repeated measurements was 6.9 years (SD=3.9). RRs derived from quintile and standardized z-score analyses were corrected by dividing the regression coefficient in the Cox model by the estimated RDR of exposure [19- 20]. RRs from the z-scores analyses which include two or more individual metabolic factors in one model were corrected by regression calibration by which each original z-score in the Cox model is replaced with its conditional expected value (20). This allows for correction of random error in measurement also for covariates. Analyses of RDR and regression calibration were based on linear mixed effect models, as described by Wood et al [20-21].

We did our analyses with both sexes combined as the number of PLC cases are few (n_{men}=195 and n_{women}=71). However, we checked for interaction between sex and each of the metabolic risk factors as continuous z-scores by including a product term of sex and the standardized factors. For significant statistical interaction, results are presented for men and women separately. Due to its high fatality, incidence and mortality are used combined as an event of interest. We included DCO cases (n=56) for whom the only source of information about the case was a death certificate.

Statistical analyses were performed in Stata (version 10.0, StataCorp LP, College Station, Texas) and R (version 2.7.2, used for random error calculation).

Results

Mean age at baseline was 43.9 years (SD=11.1) in men and 44.1 years (SD=12.3) in women (**Table 1**). Men were followed on average for 12.8 years (SD=8.6) and women for 11.3 years (SD=6.8). The prevalence of overweight or obesity (BMI 25 kg/m^2 or higher) was 55% in men and 41% in women. Among participants with a follow-up time longer than one year 266 PLC events were diagnosed. The mean age at diagnosis was 53.0 years (SD=10.9).

Increasing quintile levels of BMI and lower levels of cholesterol quintiles were significantly associated with increases in risk of PLC event (**Table 2**). The RR for the highest versus lowest quintile in models adjusted for age, smoking status and BMI; stratified by birth years, sex and sub-cohorts, and corrected for RDR, was 1.92 (95%CI 1.23 to 2.96) for BMI, and 0.23 (0.14 to 0.41) for cholesterol.

In analyses according to z-scores adjusted for age, smoking status and BMI; and stratified by birth years, sex and sub-cohorts and corrected for RDR, significant associations were found between primary liver cancer event and a unit increment of BMI (RR=1.39 95%CI, 1.24 to 1.58), blood glucose (2.13, 1.55 to 2.94) and cholesterol (0.62, 0.51 to 0.76). The RR per unit increment of the MetS z-score was 1.35 (1.12 to 1.61). In a further analysis where all the metabolic risk factors were calibrated and adjusted for each other, the association persisted only for BMI and cholesterol (**Table 3**).

Because of the strong association between alcohol consumption and liver cancer (3, 4), and a potential association between alcohol consumption and the metabolic factors investigated in our study (22), we examined alcohol consumption as a potential confounder on our studied associations. Detailed data on alcohol consumption were available in the MPP cohort, and sub-analyses in this cohort showed that adjustment of alcohol consumption did not influence risk estimates of metabolic factors and PLC (**Table 4**).

Results of sub analyses of risk according to z-scores of morphological sub types, HCC and ICC, are presented in **Fig 1.** The results for HCC were largely similar to that of PLC but the RRs for the HCC were stronger. ICC showed borderline significant association with BMI and glucose levels.

There was no significant statistical interaction between sex and z-scores of the metabolic risk factors except for blood glucose ($P_{\text{interaction}}$ = 0.02). Separate analyses of risk for men and women showed RRs of 2.67 (1.98 to 3.60) and 1.04 (0.85 to 1.29), respectively.

As liver cancer develops on the background of long-standing liver disease, we did further analyses of risk excluding cases diagnosed within the first 5 years after baseline measurement. Results showed that statistical significance of risk persisted (data not shown).

Discussion

In this large pooled European cohort study comprising 578 700 subjects and 266 primary liver cancer cases, a metabolic syndrome score, based on BMI, blood pressure, and circulating concentrations of glucose, total cholesterol, and triglycerides, was significantly associated with primary liver cancer risk. Further analysis of single metabolic risk factors revealed that BMI and glucose were significantly associated with increased risk of PLC.

Our findings for BMI are in accordance with results of most previous prospective reports that have shown the risk of liver cancer to be twice as high among obese subjects as in non obese controls [23-26]. Furthermore, the current study showed that the association between BMI and PLC could be an independent effect as the relative risk of PLC remained statistically significant even after adjusting the model for the rest of metabolic syndrome factors. Considering studies which reported that up to 90% obese individuals have some degree of fatty changes in the liver, the observed association between excess body weight and increased risk of PLC in our study, appears to be supportive of reports that liver cancer in obese individuals may be mediated through the development of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). In addition, NASH predisposes to lipid peroxidation and excess free radical activity with the potential risk of genomic mutations [27].

The role of high blood pressure in cancer incidence and mortality is unclear. A meta-analysis on the association between hypertension and cancer mortality indicated that hypertension is associated with an increased risk of cancer mortality [28]. The underlying mechanisms however have not been defined, although increase in cell proliferation has been proposed [29]. We found a 2.8-fold higher risk in the top quintile of mean blood pressure compared to the lowest quintile, however, the association became non-significant after adjustment for BMI. These observations do not support an independent role of blood pressure as risk factor for PLC.

Our study suggests that high blood glucose level is associated with PLC in men. Similar results have been reported from several other cohort or case control studies [30]. Further evidence comes from studies which have shown diabetes to be associated with a spectrum of liver diseases ranging from non-alcoholic fatty liver diease (NAFLD), non-alcoholic steatohepatitis (NASH) and cirrhosis [31, 32]. According to a recent meta-analysis on the association between hepatocellular carcinoma and diabetes, the majority of the studies showed a significantly increased risk with a pooled risk ratio of 2.5 (95% CI 1.9 to 3.2) [30]. In several of these

studies information on alcohol consumption, smoking or viral hepatitis was available, but adjustment for these factors resulted in either no or minimal change in the risk estimates [33-36]. Similarly, few studies had accounted for BMI in their analyses, and found that body mass index had no effect on the association between diabetes and liver cancer [34, 37]. In our study, blood glucose in men showed a positive association with risk also after adjustment for BMI as shown in table 3.

Underlying chronic liver disease causing diabetes is thought to explain the association between blood glucose levels and PLC [13]. To account for the time lag between liver disease and cancer incidence we performed a sub analysis excluding the first 5 years of follow up after the baseline measurement which revealed that the effect of blood glucose levels on the risk of PLC remained the same. This provides evidence that diabetes precedes PLC; however it is difficult to conclude if it precedes chronic liver diseases.

Our study revealed a strong significant inverse association between total cholesterol level and PLC risk. A recent study on total serum cholesterol and cancer incidence revealed that the reverse association may be largely due to preclinical effects of cancer on total serum cholesterol but it failed to prove this effect on cancers of the gastrointestinal tract [38]. Moreover there are experimental studies which showed that cholesterol is increasingly accumulated in hepatic tumor cells as the tumor cells consume this lipid for their growth [39,40] eventually decreasing the serum level. Lag time analysis in our study, however, showed that the significant inverse association persisted even after excluding the first 5 years of follow up after baseline measurement.

The relative risks of HCC by exposure variables were slightly higher than the risk seen with both subtypes combined. This may indicate that metabolic factors as risk for PLC are more relevant for the occurrence of HCC. On the other hand, the lack of significant associations between ICC and metabolic risk factors is likely due the small number of ICC cases (n=28) in our study. However, despite the small number of cases, the borderline significant association between blood glucose and ICC is worthy of note. There are no studies, to date, which indicated the possible role of high blood glucose as risk factor for ICC except for a speculative report on diabetes, insulin resistance and ICC [41].

The main strength of our study is the large data set from seven prospective cohorts with high quality cancer registries with almost complete coverage of cases and data from cause of death registries which gave us high power to detect even quite modest associations as well as

associations in lag time analyses. In addition, the large number of repeated measurements allowed us to correct for random error in exposure variables.

Limitations of the study are lack of data on hepatitis virus infection status, which, in some studies, is assumed to be a potential confounder. However, this assumption is controversial and it is unlikely that hepatitis viral infection is related to metabolic risk factors and thus, unlikely to affect the findings of this study [26, 42]. Moreover, several case-control and cohort studies showed no or minimal changes in risk after further adjustment for hepatitis infection on the association between obesity or diabetes and liver cancer [26, 30]. Another limitation of the study is that there is a slight difference in measurement methods in the sub-cohorts [16]. However, in our analyses, we tried to overcome this problem by using cohort specific cut-points in the analysis of exposures by quintiles and by standardization with z-scores. Additionally we stratified for cohorts in all the analyses.

In summary, our study showed that major metabolic risk factors are significantly associated with risk of primary liver cancer. Whereas, BMI and glucose are associated with increased risk, cholesterol showed an inverse association. Beyond the individual factors, the results of our study show, for the first time, that the metabolic syndrome as an entity presents a significant risk constellation for the occurrence of liver cancer.

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Table 1. Baseline characteristics of study participants in the Metabolic syndrome and Cancer project (Me-Can)

	Men	Women
Cohort (year of baseline measurement), *n* participants (%)		
Oslo (1972-73)	16 760 (6)	
NCS (1974-83)	25 952 (9)	25 072 (9)
CONOR (1995-2003)	52 181 (18)	57 687 (20)
40-y (1994-99)	60 676 (21)	68 211 (23)
VHM&PP (1988-2002)	73 213 (25)	86 671 (30)
VIP (1985-2005)	38 843 (13)	40 669 (14)
MPP (1974-92)	22 241 (8)	10 524 (4)
Total (1972-2005)	289 866	288 834
Baseline age, years		
Mean (SD)	43.9 (11.1)	44.1 (12.3)
Categories, *n* (%)		
<30	27 244 (9)	33 067 (11)
30- <45	157 145 (54)	154 462 (54)
45- <60	76 623 (27)	67 689 (23)
60-	28 854 (10)	33 616 (12)
Fasting time, hours, *n* (%)[1]		
<4	120 510 (41)	122 319 (42)
4-8	30 769 (11)	26 802 (9)
>8	138 587 (48)	139 713 (49)
Smoking status, *n* (%)		
Never smoker	113 496 (39)	144 815 (50)
Ex-smoker	86 086 (30)	72 600 (25)
Current smoker	89 419 (31)	70 721 (25)
Missing	865 (0)	698 (0)
BMI, kg/m^2		
Mean (SD)	25.7 (3.5)	24.9 (4.4)
Categories, *n* (%)		
<25	131 167 (45)	170 535 (59)
25- <30	127 846 (44)	82 869 (29)
30-	30 853 (11)	35 430 (12)
Follow-up, years		
Mean (SD)	12.8 (8.6)	11.3 (6.8)
Categories, *n* (%)		
<5	36 755 (13)	35 451 (12)
5 - <15	178 968 (62)	199 151 (69)
15 - <25	24 971 (8)	29 751 (10)
25-	48 172 (17)	24 481 (9)

[1]Proportion of participants with a fasting time >8 h: 5% in the Norwegian cohorts, 90% in the VIP, and 100% in the VHM&PP and MPP.

Abbreviations: Oslo=Oslo study I; NCS=Norwegian Counties Study; CONOR=Cohort of Norway; 40-y=Age 40-programme; VHM&PP=Vorarlberg Heath Monitoring and Prevention Programme; VIP=Västerbotten Intervention Project; MPP=Malmö Preventive Project; SD=standard deviation; BMI=body mass index.

Table 2. Risk of Primary liver cancer in relation to quintiles of metabolic factors

Exposures	Quintile level [1]	Primary liver cancer (n=266) Mean (SD)	*n*, cases	Model 1 [2]	Model 2 [3]
BMI (kg/m^2)	**1**	20.7 (1.5)	36	1.00	
	2	23.0 (1.1)	38	0.91(0.55-1.51)	
	3	24.7 (1.0)	45	0.97(0.59-1.57)	
	4	26.8 (1.0)	53	1.02(0.63-1.64)	
	5	31.3 (3.2)	94	1.92(1.23-2.96)	
	***P* trend**			0.001	
Mid BP (mmHg)	**1**	88.2 (5.7)	29	1.00	1.00
	2	97.0 (4.1)	40	1.40(0.59-3.38)	1.33(0.55-3.16)
	3	102.7 (3.8)	41	1.25(0.52-3,04)	1.13(0.47-2.74)
	4	109.8 (4.1)	57	1.51(0.65-3.48)	1.25(0.53-2.95)
	5	124.5(10.4)	99	2.80(1.27-6.17)	2.08(0.95-4.73)
	***P* trend**			0.006	0.07
Glucose (mmol/l)	**1**	4.1 (0.5)	47	1.00	1.00
	2	4.7 (0.3)	41	0.60(0.14-2.51)	0.55(0.13-2.38)
	3	5.1 (0.3)	52	1.43(0.38-5.61)	1.30(0.33-5,05)
	4	5.5 (0.4)	44	0.75(0.18-3.14)	0.65(0.16-2.71)
	5	6.7 (1.9)	82	3.88(1.11-13.5)	2.78(0.78-9.96)
	***P* trend**			0.02	0.08
Cholesterol (mmol/l)	**1**	4.2 (0.5)	62	1.00	1.00
	2	5.0 (0.3)	42	0.34(0.18-0.62)	0.33(0.18-0.61)
	3	5.6 (0.3)	48	0.32(0.18-0.58)	0.31(0.17-0.54)
	4	6.2 (0.3)	55	0.29(0.17-0.52)	0.27(0.15-0.47)
	5	7.4 (0.8)	59	0.26(0.14-0.46)	0.23(0.14-0.41)
	***P* trend**			<0.001	<0.001
Triglycerides[§] (mmol/l)	**1**	0.7 (0.2)	41	1.00	1.00
	2	1.0 (0.2)	45	0.84(0.33-2,06)	0.76(0.31-1,91)
	3	1.3(0.3)	42	0.55(0.22-1.40)	0.48(0.19-1.23)
	4	1.8 (0.4)	69	1.35(0.55-3,09)	1,02(0.44-2,43)
	5	3.1 (1.5)	63	0.90(0.37-2.13)	0.59(0.24-1.43)
	***P* trend**			0.70	0.51

[1] Quintile levels grouped by cohort and sex and for glucose, cholesterol and triglycerides further by fasting time.
[2] RR were estimated from Cox regression models with attained age as time scale adjusted for smoking status and age at baseline, stratified by cohort, categories of birth year and sex. RRs are corrected for regression dilution bias by use of the regression dilution ratio (RDR); conversion into uncorrected RR = exp(log(RR)*RDR). RDR: BMI, 0.90; mean blood pressure, 0.54; log(glucose), 0.28; cholesterol, 0.66; log(triglycerides), 0.51. Glucose and triglycerides were logarithmically transformed.
[3] RR were further adjusted for quintiles levels of BMI (except in BMI analysis)
[§] value missing for 6 cases
Abbreviation: RR, relative risk; SD, standard deviation; BMI, body mass index; Mid BP, mean blood pressure; RDR, regression dilution ratio

Table 3. Relative risk (95% CI) of primary liver cancer, by z-scores of metabolic factors, and of the MetS score.

Exposure	Primary liver cancer (n= 266)		
	Model [1]	Model [2]	Model [3]
BMI	1.39(1.24-1.58)		1.21(1.03-1.42)
Mean blood pressure	1.29(1.06-1.60)	1.08 (0.86-1.36)	1.03(0.81-1.30)
Glucose[4]	2.38(1.76-3.14)	2.13 (1.55-2.94)	1.24(0.45-3.37)
Cholesterol	0.67(0.56-0.82)	0.62 (0.51-0.76)	0.65(0.52-0.82)
Triglycerides[4]	1.09(0.84-1.43)	0.85 (0.65-1.10)	1.13(0.90-1.42)
MetS	1.35(1.12-1.61)		

[1] Relative risk were estimated from Cox regression models, with attained age as time scale, stratified by cohort, birth year and sex; adjusted for baseline age, smoking status and BMI and were corrected for regression dilution bias by use of regression dilution ratio (RDR); conversion into uncorrected RR = exp(log(RR)*RDR). RDR: BMI, 0.90; mean blood pressure, 0.54; log(glucose), 0.28; cholesterol, 0.66; log(triglycerides), 0.51.
[2]RRs were further adjusted for BMI except for Mets score analysis. In addition, z-scores, derived from original values, were corrected for regression dilution bias by calibration.
[3] Relative risks were further adjusted for all the individual z-scores (except in MetS score analysis). In addition, z-scores, derived from original values, were calibrated.
[4] Glucose and triglycerides were logarithmically transformed.
Abbreviations: CI, confidence interval; MetS, metabolic syndrome; BMI, body mass index; RR, relative risk

Table 4. Relative risk (95% CI) of primary liver cancer, by z-scores of metabolic factors and of the MetS score in the Malmö Preventive Project (MPP) with and without adjustment for alcohol consumption.

	Primary liver cancer (n=55)	
Exposure	**Model 1[1]**	**Model 2[2]**
BMI	1.71(1.34-2.17)	1.70(1.34-2.16)
Mean blood pressure	1.31(0.83-2.08)	1.32(0.84-2.09)
Glucose[3]	1.01(0.41-2.51)	1.13(0.46-2.80)
Cholesterol	0.90(0.59-1.38)	0.87(0.55-1.34)
Triglycerides[3]	0.96(0.55-1.66)	0.98(0.56-1.69)
MetS	1.68(1.16-2.41)	1.70(1.18-2.43)

[1] Relative risk were estimated from Cox regression models, with attained age as time scale, stratified by cohort, birth year and sex; adjusted for baseline age, smoking status and BMI. In addition , z-scores, derived from original values, were corrected for regression dilution bias by calibration (except BMI and Mets score analyses).
[2]Relative risks were further adjusted for alcohol consumption.
[3]Glucose and triglycerides were logarithmically transformed.
Abbreviations: CI, confidence interval; MetS, metabolic syndrome; BMI, body mass index; RR, relative risk

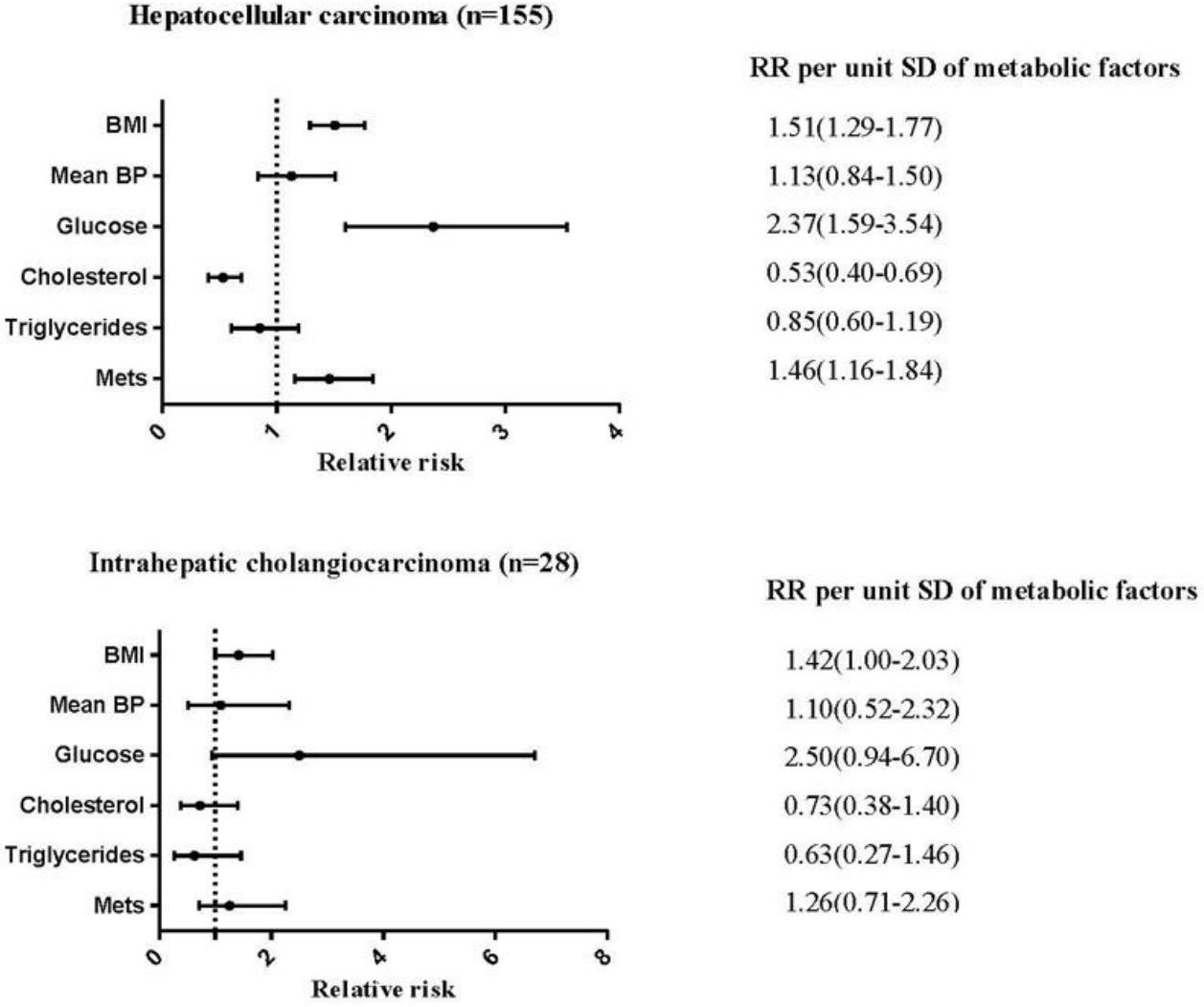

Fig1. Risk of morphological subtypes of primary liver cancer by z-scores of metabolic factors, and of the MetS score[1].

[1]Relative risk were estimated from Cox regression models, with attained age as time scale, stratified by cohort, birth year and sex; adjusted for baseline age, smoking status and BMI. In addition , z-scores, derived from original values, were corrected for regression dilution bias by calibration (except BMI and Mets score analyses).
Glucose and triglycerides were logarithmically transformed.
HCC and ICC do not sum up to 266 as 83 cases of PLC are not classified to their morphological subtypes.
Abbreviations: MetS, metabolic syndrome; BMI, body mass index; RR, relative risk; SD, standard deviation; Mean BP, mean blood pressure

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A Prospective Study on Metabolic Risk Factors and Gallbladder Cancer in the Metabolic Syndrome and Cancer (Me-Can) Collaborative Study

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Abstract

Objective: To investigate the association between metabolic risk factors (individually and in combination) and risk of gallbladder cancer (GBC).

Methods: The metabolic syndrome and cancer project (Me-Can) includes cohorts from Norway, Austria, and Sweden with data on 578,700 men and women. We used Cox proportional hazard regression models to calculate relative risks of GBC by body mass index (BMI), blood pressure, and plasma levels of glucose, cholesterol, and triglycerides as continuous standardised variables and their standardised sum of metabolic syndrome (MetS) z-score. The risk estimates were corrected for random error in measurements.

Results: During an average follow-up of 12.0 years (SD = 7.8), 184 primary gallbladder cancers were diagnosed. Relative risk of gallbladder cancer per unit increment of z-score adjusted for age, smoking status and BMI (except for BMI itself) and stratified by birth year, sex and sub-cohorts, was for BMI 1.31 (95% confidence interval 1.11, 1.57) and blood glucose 1.76 (1.10, 2.85). Further analysis showed that the effect of BMI on GBC risk is larger among women in the premenopausal age group (1.84 (1.23, 2.78)) compared to those in the postmenopausal age group (1.29 (0.93, 1.79)). For the other metabolic factors no significant association was found (mid blood pressure 0.96 (0.71, 1.31), cholesterol 0.84 (0.66, 1.06) and serum triglycerides 1.16 (0.82, 1.64)). The relative risk per one unit increment of the MetS z-score was 1.37 (1.07, 1.73).

Conclusion: This study showed that increasing BMI and impaired glucose metabolism pose a possible risk for gallbladder cancer. Beyond the individual factors, the results also showed that the metabolic syndrome as an entity presents a risk constellation for the occurrence of gallbladder cancer.

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Introduction

Primary gallbladder cancer (GBC) is the most common biliary tract tumour and the sixth most common cancer affecting the gastrointestinal tract [1,2]. It is a disease typically characterised by late diagnosis and poor outcome with a five year survival of only about 32% [3]. Although the presence of gallstones is considered to be an important risk factor, several other unidentified factors may be important in the development of gallbladder carcinoma. About 10 to 25% of patients with this disease do not have associated cholelithiasis and only a small proportion (1 to 3%) of patients that do have gallstones actually develop cancer [4].

Metabolic syndrome (MetS) is a constellation of factors related to insulin resistance including obesity, impaired glucose tolerance, dyslipidaemia and hypertension with varying definitions [5]. It has consistently been associated with an increased risk of cardiovascular diseases and diabetes type 2 [6,7], and recently with risk of cancer at some sites like colorectal, prostate and liver cancers

[8 13]. There is little data on the association between the MetS and risk of GBC, for separate as well as for a combination of MetS factors [10 17]. Most of these studies are either based on a single specific metabolic factor like obesity or diabetes [10 12,14], apply an unfavourable proxy for MetS or they are non-prospective in nature [13 17]. To our knowledge this is the largest prospective study that assessed MetS and separate metabolic risk factors like serum lipids and blood pressure in association with gallbladder carcinoma.

In this large study of 578,700 participants, we aimed to investigate the association between metabolic risk factors, individually and in combination, and the risk of gallbladder cancer, taking random error into account.

Materials and Methods

Detailed description of materials and methods of this study has been presented previously [18,19].

Study Population and Measurements

The study population comes from the Metabolic syndrome and Cancer project (Me-Can) which includes cohorts with 578,700 participants from Norway, Austria and Sweden. In these cohorts, health examinations data have been collected on height, weight, blood pressure, blood levels of glucose, total cholesterol, triglycerides, and smoking status. Time period of data collection spanned from 1972 to 2006. A detailed description of Me-Can and inclusion criteria for participants in this study has been previously described [18].

Follow-up and Endpoints

Linkages have been performed with cause of death and vital status registries of the respective countries in order to identify those cases with incident gallbladder cancer (ICD-7): 155.1). Endpoints for the study were set at the date of the first cancer diagnosis, emigration, death, or December 31, 2003 (Austria), 2005 (Norway) and 2006 (Sweden).

Statistical Analysis

The statistical analysis of this study is similar to a previously published study by the same study group [19]. In brief, Cox proportional hazards regression models, with age as the time variable, were fitted to obtain hazard ratios, denoted as relative risks (RRs), of primary GBC incidence with 95% confidence intervals (95% CI). We did our main analyses with both sexes combined as there was no significant interaction between sex and each of the MetS factors. As in the previous publications of Me-Can studies, analyses were undertaken with exposures as quintiles, standardized z-score continuous variables as well as bi-categorical values using the WHO defined cut-off points of the determinant variables.

Quintile Analysis

Quintile cut-off points for the exposure variables were calculated within each cohort and sex. For glucose, cholesterol and triglycerides, cut-offs were additionally stratified by fasting time before blood sampling (>8 hours, fasting or ≤8 hours, non-fasting). The models were further stratified for the seven cohorts, sex and year of birth (five categories: ≤1929, 1930 39, 1940 49, 1950 59, and ≥1960), and adjusted for age, smoking status (three categories: never, former and current smokers) and for BMI where appropriate. The lowest quintile was used as a reference. Mean levels within the quintiles of exposure variables were used to test for linear trend.

Standardized z-score Analysis

In addition to the quintile analysis, we also performed statistical tests with the exposures on a continuous scale. Standardized scores let each determinant to be investigated in the same scale making a uniform comparison possible. We transformed the existing values to standardised variables (z-scores), with zero as mean and one as standard deviation ($z = (x - \mu)/\sigma$). As in the quintile analysis, the transformation is stratified by cohort, sex, and fasting time. Skewed variables (glucose and triglycerides) were logarithmically transformed prior to standardisation. Metabolic syndrome (MetS) score was constructed by adding the individual z-scores, and further standardization of the resulting sum. The adjustments and the stratifications in the z-score analysis are the same as in the quintile analysis.

Analysis by WHO Standards

We also estimated risks in two categories according to cut-offs defined by WHO as follows: overweight (BMI 25 <30 kg/m^2), obesity (BMI ≥30 kg/m^2), hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥90 mmHg), impaired glucose tolerance (fasting glucose 6.0 6.9 mmol/l), diabetes (fasting glucose ≥7.0 mmol/l), hypertriglyceridemia (fasting triglycerides ≥1.7 mmol/l), and hypercholesterolemia (fasting total cholesterol ≥6.2 mmol/l). For blood glucose and lipids only those individuals who had fasted >8 hours prior to blood draw were included [20 22]. The same adjustment and stratification scheme was used as in the models with quintile and continuous exposure variables.

Random Errors

All risk estimates were adjusted for random error in exposure measurements, based on data on repeated measurements from 133,820 participants with a total of 406,364 observations. These data were used to estimate regression dilution ratios (RDR) or regression calibration (RC) based on linear mixed effect models [23 25]. RRs derived from quintile and standardised z-score analyses were then corrected by dividing the regression coefficient in the Cox model by the estimated regression dilution ratio (RDR) of exposure. RRs from the z-scores analyses which adjusted for all individual metabolic factors in one model were corrected by regression calibration.

Further Analytic Considerations

Since reproductive factors are important risk factor for gallbladder diseases in women [3,4], we did additional risk estimation separately for women <50 years of age (n = 214,572) and ≥50 years of age (n = 72,748) using this age-cut-off as a proxy for pre- and postmenopausal status, respectively.

Our main analyses excluded the first year after baseline measurements in order to account for possible reverse causality between exposures and event. We consolidated the issue by performing further lag-time analyses that excluded the first 3 years of follow-up.

Statistical analyses were performed in Stata (version 10.0, StataCorp LP, College Station, Texas) and R (version 2.7.2, used for random error correction).

Ethics

The study was approved by The Research Review Board of Umeå, Sweden, the Regional Committee for Medical and Health Research Ethics, Southeast Norway and the Ethikkommission of the Land Vorarlberg, Austria. Participants from Sweden and Austria provided written informed consent to participate in this

study. In Norway, the participants were invited to come to the health survey and a questionnaire was sent together with the invitation. An attendance to the health examination where the participants delivered their filled in questionnaire, has been accepted by the Data Inspectorate as an informed consent, but not a written consent. Written consent was obtained from 1994 onwards.

Results

Mean age at baseline was 43.9 years (SD = 11.1) in men and 44.1 years (SD = 12.3) in women (Table 1). Men were followed on average for 12.8 years (SD = 8.6) and women for 11.3 years (SD = 6.8). The prevalence of overweight or obesity (BMI 25 kg/m^2 or higher) was 55% in men and 41% in women. Among participants with a follow-up time longer than one year 91 men and 93 women were diagnosed with primary GBC. Mean ages at the time of cancer diagnosis were 62.9 years (SD = 8.7) in men and 65.5 years (SD = 10.9) in women.

In quintile analysis, BMI and blood glucose were significantly associated with increases in risk of GBC (Table 2). The relative risk for the highest versus lowest quintile in models, adjusted for age, smoking status and BMI (except for BMI itself), stratified by birth years, sex and cohorts, and corrected for RDR, was 1.94 (95% CI 1.08, 3.51) for BMI and 5.38 (1.11, 26.5) for blood glucose.

In multivariable adjusted analyses of z-scores, significant associations were found for a unit z-score increment of BMI (1.31 (1.11, 1.57)) and blood glucose (1.76 (1.10, 2.85)). The relative risk per unit increment of the MetS z-score was 1.37 (1.07, 1.73). In a further analysis where all the metabolic risk factors were calibrated and adjusted for each other, the significant association persisted only for BMI. No statistically significant association with GBC was observed for blood pressure, cholesterol and triglycerides (Table 3).

There were no statistically significant interactions when testing effect modification of metabolic factors on GBC risk. Notable were however varying associations of BMI with GBC by age. The relative risk per unit increment of BMI was 1.84 (1.23, 2.78) in premenopausal (n = 32) and 1.29 (0.93, 1.79) (n = 61) in postmenopausal (≥50 years of age) women.

In analyses of the exposures in dichotomised categories according to the WHO classification of risk factors (Table 4), increases in risk were found for individuals with overweight (BMI above versus below 25 kg/m^2) and individuals with impaired glucose metabolism (fasting blood glucose above versus below 6.0 mmol/l) with a relative risk of 1.52 (1.12, 2.10) and 1.62 (1.00, 2.62), respectively. These analyses were restricted to 278,300 individuals with >8 h fasting time.

Supplementary tables S1 S4 show sub-analyses of risks for men and women separately. Sex-specific risk estimates were similar to the combined analyses with some exceptions. Notably, the magnitude of the observed association between BMI and GBC was stronger and statistically significant in women.

Discussion

In this large cohort study comprising of 578,700 men and women, a composite metabolic syndrome score, based on BMI, blood pressure, and circulating concentrations of glucose, total cholesterol and triglycerides, was significantly associated with GBC risk. Further analysis of single metabolic risk factors revealed that BMI and glucose were significantly associated with increased risk of GBC.

Table 1. Baseline characteristics of study participants in the Metabolic syndrome and Cancer project (Me-Can) including the first year of follow-up (n = 578,700).

Cohort (year of baseline measurement), n participants (%)	Men	Women
Oslo (1972–73)	16,760 (6)	
NCS (1974–83)	25,952 (9)	25,072 (9)
CONOR (1995–2003)	52,181 (18)	57,687 (20)
40-y (1994–99)	60,676 (21)	68,211 (23)
VHM&PP (1988–2002)	73,213 (25)	86,671 (30)
VIP (1985–2005)	38,843 (13)	40,669 (14)
MPP (1974–92)	22,241 (8)	10,524 (4)
Total (1972–2005)	289,866	288,834
Baseline age, years		
Mean (SD)	43.9 (11.1)	44.1 (12.3)
Categories, n (%)		
<30	27,244 (9)	33,067 (11)
30– <45	157,145 (54)	154,462 (54)
45– <60	76,623 (27)	67,689 (23)
60-	28,854 (10)	33,616 (12)
Fasting time, hours, n (%)1		
<4	120,510 (41)	122,319 (42)
4–8	30,769 (11)	26,802 (9)
>8	138,587 (48)	139,713 (49)
Smoking status, n (%)		
Never smoker	113,496 (39)	144,815 (50)
Ex-smoker	86,086 (30)	72,600 (25)
Current smoker	89,419 (31)	70,721 (25)
Missing	865 (0)	698 (0)
BMI, kg/m2		
Mean (SD)	25.7 (3.5)	24.9 (4.4)
Categories, n (%)		
<25	131,167 (45)	170,535 (59)
25– <30	127,846 (44)	82,869 (29)
30–	30,853 (11)	35,430 (12)
Follow-up, years		
Mean (SD)	12.8 (8.6)	11.3 (6.8)
Categories, n (%)		
<5	36,755 (13)	35,451 (12)
5 – <15	178,968 (62)	199,151 (69)
15 – <25	24,971 (8)	29,751 (10)
25–	48,172 (17)	24,481 (9)

[1]Proportion of participants with a fasting time >8 h: 5% in the Norwegian cohorts, 90% in the VIP, and 100% in the VHM&PP and MPP.
Abbreviations: Oslo = Oslo study I; NCS = Norwegian Counties Study; CONOR = Cohort of Norway; 40-y = Age 40-programme; VHM&PP = Vorarlberg Heath Monitoring and Prevention Programme; VIP = Västerbotten Intervention Project; MPP = Malmö Preventive Project; SD = standard deviation; BMI = body mass index.
doi:10.1371/journal.pone.0089368.t001

Table 2. Risk of Primary gallbladder cancer in relation to quintiles of metabolic factors (n = 575,390).

		Primary gallbladder cancer (n = 184)					
		Mean (SD)	n, cases	Model[2]		Model[3]	
Exposures	Quintile level[1]			RR	95%CI	RR	95%CI
BMI	1	20.7 (1.3)	20	1.00			
(kg/m2)	2	23.0 (0.8)	26	1.12	0.58, 2.19		
	3	24.7 (0.8)	38	1.49	0.80, 2.76		
	4	26.8 (0.9)	47	1.70	0.93, 3.09		
	5	31.3 (2.6)	53	1.94	1.08, 3.51		
	P_{trend}			0.08			
Mean BP[§]	1	8.2 (4.9)	20	1.00		1.00	
(mmHg)	2	96.9 (2.4)	27	1.37	0.48, 4.01	1.27	0.44, 3.74
	3	102.7 (2.3)	41	2.11	0.77, 5.75	1.86	0.68, 5.04
	4	109.8 (2.9)	35	1.02	0.36, 2.86	0.82	0.29, 2.32
	5	124.5 (9.5)	60	1.81	0.68, 4.81	1.25	0.45, 3.45
	P_{trend}			0.47		0.92	
Glucose	1	4.2(0.5)	31	1.00		1.00	
(mmol/l)	2	4.8 (0.3)	34	2.51	0.47, 13.5	2.32	0.43, 12.7
	3	5.1 (0.3)	28	1.18	0.20, 7.11	1.07	0.18, 6.33
	4	5.5 (0.4)	38	3.14	0.60, 16.5	2.64	0.49, 13.9
	5	6.8 (2.0)	53	7.52	1.56, 36.1	5.38	1.11, 26.5
	P_{trend}			0.01		0.04	
Cholesterol	1	4.2 (0.5)	27	1.00		1.00	
(mmol/l)	2	5.0 (0.3)	37	1.14	0.53, 2.47	1.11	0.52, 2.38
	3	5.6 (0.3)	34	0.74	0.33, 1.61	0.70	0.32, 1.53
	4	6.2 (0.3)	40	0.71	0.35, 1.53	0.66	0.31, 1.42
	5	7.4 (0.8)	46	0.67	0.32, 1.46	0.62	0.29, 1.32
	P_{trend}			0.14		0.08	
Triglycerides[§§]	1	0.7 (0.2)	22	1.00		1.00	
(mmol/l)	2	1.0 (0.2)	30	1.48	0.45, 4.88	1.38	0.38, 2.00
	3	1.3 (0.3)	34	1.40	0.44, 4.49	1.20	0.21, 1.16
	4	1.9 (0.4)	46	2.50	0.84, 7.61	1.94	0.40, 1.97
	5	3.1 (1.7)	48	2.06	0.67, 6.28	2.90	0.30, 1.53
	P_{trend}			0.12		0.50	

[1]Quintile levels grouped by cohort and sex and for glucose, cholesterol and triglycerides further by fasting time. RRs were estimated from Cox regression models with attained age as time scale after excluding the first year after baseline measurement.
[2]RRs were adjusted for smoking status and age at baseline, stratified by cohort, sex and categories of birth year. RRs are corrected for regression dilution bias by use of the regression dilution ratio (RDR); conversion into uncorrected RR = exp (log (RR)*RDR). RDR: BMI, 0.90; mean blood pressure, 0.54; glucose, 0.28; cholesterol, 0.66; triglycerides, 0.51. Glucose and triglycerides were logarithmically transformed.
[3]RR were further adjusted for quintiles levels of BMI (except in BMI analysis).
[§]value missing for 1 case.
[§§]value missing for 4 cases.
Abbreviations: RR, relative risk; SD, standard deviation; BMI, body mass index; Mid BP, mean blood pressure; RDR, regression dilution ratio.
doi:10.1371/journal.pone.0089368.t002

Strengths and Limitations

The main strengths of our study are the large number of participants and its prospective design. We used data from population-based surveys in three countries, with almost complete coverage of data for measured exposure factors. The large number of repeated measurements within the study population allowed us to adjust for random error in the individual MetS factors. We also used high quality national registers in Austria, Norway, and Sweden for the follow-up regarding cancer diagnoses [26 28].

An important limitation of the study is the lack of data on gallstone status - a well established risk factor for GBC [3,4,29]. Gallstone status is also highly linked to the presence of metabolic risk factors like obesity, diabetes and dyslipidemia [17,30]. With this constellation we cannot exclude the possible mechanistic role of gallstones in the association between metabolic risk factors and GBC as has been elegantly presented in previous prospective studies on obesity and diabetes [31,32] as well as case-control studies [15 16]. However, it is also evident that a considerable proportion of individuals with GBC show no sign of cholelithiasis [3,4], signifying the presence of other factors that may play important role gallbladder carcinogenesis.

Another limitation of the study is the noticeably small number of events, despite the large number participants, which might have

Table 3. Relative risk (95% CI) of primary gallbladder cancer, by z-scores of metabolic factors, and of the MetS score (n = 575,390).

	Primary gallbladder cancer (n = 184)					
	Model[1]		Model[2]		Model[3]	
Exposures	RR	95%CI	RR	95%CI	RR	95%CI
BMI	1.31	1.11, 1.57			1.23	1.03, 1.46
Mean blood pressure	1.10	0.82, 1.46	0.96	0.71, 1.31	0.97	0.82, 1.15
Glucose[4]	1.97	1.38, 3.22	1.76	1.10, 2.85	1.58	0.98, 2.54
Cholesterol	0.87	0.69, 1.11	0.84	0.66, 1.06	0.84	0.64, 1.10
Triglycerides[4]	1.14	0.98, 1.88	1.16	0.82, 1.64	1.11	0.77, 1.61
MetS	1.37	1.07, 1.73				

[1]Relative risks were estimated from Cox regression models after excluding the first year of follow-up after baseline measurement, with attained age as time scale, stratified by cohort, sex and categories of birth year, adjusted for baseline age and smoking status, and corrected for regression dilution bias by use of regression dilution ratio (RDR); conversion into uncorrected RR = exp (log (RR)*RDR). RDR: BMI, 0.90; mean blood pressure, 0.54; log(glucose), 0.28; cholesterol, 0.66; log(triglycerides), 0.51.
[2]Additionally adjusted for BMI.
[3]Further adjusted for all the individual z-scores (except in MetS score analysis). In addition, z-scores, derived from original values, were calibrated.
[4]Glucose and triglycerides were logarithmically transformed.
Abbreviations: CI, confidence interval; MetS, metabolic syndrome; BMI, body mass index; RR, relative risk.
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Table 4. Relative risk (95% CI) of primary gallbladder cancer by WHO categories of metabolic factors (n = 575,390).

Exposures		Relative risks (95% CI)				
		n, cases	Model[2]		Model[3]	
	Cut-off levels[1]		RR	95%CI	RR	95%CI
BMI	<25	77	1.00			
(kg/m^2)	≥25	107	1.52	1.12, 2.10		
Systolic BP	<140	108	1.00		1.00	
(mmHg)	≥140	76	1.04	0.75, 1.44	0.93	0.66, 1.29
Diastolic BP	<90	123	1.00		1.00	
(mmHg)	≥90	61	1.00	0.73, 1.37	0.96	0.74, 1.25
Fasting glucose[4] (mmol/l)	<6.0	101	1.00		1.00	
	≥6.0	23	1.80	1.12, 2.88	1.62	1.00, 2.62
Fasting total cholesterol[4]	<6.2	78	1.00		1.00	
(mmol/l)	≥6.2	46	0.90	0.62, 1.32	0.89	0.61, 1.29
Fasting triglycerides[4]	<1.7	80	1.00		1.00	
(mmol/l)	≥1.7	44	1.22	0.83, 1.80	1.09	0.73, 1.62

[1]Cut-off levels are according to WHO definition.
[2]RRs were estimated from Cox regression models with attained age as time scale, adjusted for smoking status and age at baseline, stratified by cohort, categories of birth year, and sex.
[3]RRs were further adjusted for BMI (except in BMI analysis).
[4]RRs were estimated only for individuals who had fasted 8 or more hours before baseline blood sampling (n = 277,300).
Abbreviations: BMI, body mass index; BP, blood pressure; CI, confidence interval; RR, relative risk.
doi:10.1371/journal.pone.0089368.t004

contributed to the large confidence intervals seen especially in the quintile analyses. Lack of information on the reproductive history [3] among women may also be a limitation. However, we did a further risk estimation for women <50 and ≥50 years of age as a proxy for pre- and postmenopausal age groups, respectively. Our study is also limited by lack of data on socioeconomic status as well as some other behavioural aspects like alcohol consumption and physical activity. Moreover we lack data on treatment history of the abnormal metabolic factors like hypertension and dyslipidemia which might, to some extent, have confounded our risk estimate. For the most recent definition of the MetS [5] we lack data on specific factors like waist circumference and high density lipoprotein cholesterol which we had to replace with BMI and total cholesterol respectively. Consequently we have presented our results based on a metabolic syndrome score which we used as a proxy for the syndrome [33].

Comparisons with the Literature

The observed significant association between metabolic syndrome and GBC in this study with large number of participants and adequately long follow-up period strengthens reports by a previous case-control study based on over 600 biliary tract cancers which also found significant role of Mets on GBC [17]. However, such studies may be limited by the timing of exposure and outcome not being able to exclude reverse causality. This may probably be the case with the lack of association between waist circumference and risk of GBC. Such deficiencies are better dealt with prospective studies of long follow up period. Although several prospective studies exist on individual metabolic factors [31,32], literatures on the association between MetS as an entity and GBC are scarce [13]. In a previous prospective study [13] MetS was defined as simultaneous exposure to antihypertensive, hypoglycaemic and hypolypemic treatments which is a rather rough approximation of MetS. Compared to ours this previous study did not find statistically significant association between MetS and GBC which is a rather questionable finding in the face of evident significant association with most of the individual components.

Although several independent mechanisms are depicted to underpin the association between obesity and cancer, the mechanisms that link obesity with gallbladder cancer risk are unclear [34 36]. Many studies have identified obesity as a risk to be more pronounced in women, and suggested a possible role of sex hormones (mainly oestrogen) in the pathogenesis of GBC [8,13,29,36 41]. There are even indications that sex hormone receptors exist on the tumour tissue [42]. Our results, that increasing BMI poses greater risk mainly in younger women of premenopausal age group, might be supportive of this mechanism. This finding is supported by another cohort study in Norway [13].

In tumours that depend on oestrogen for their growth, like breast and endometrial cancers, obesity is shown to be of greater risk in postmenopausal women [43 46]. In our study, however, the risk for GBC was higher for younger women below the age of 50. This observation might be due to chance, as we did not find a significant interaction between age and BMI. However, if confirmed in further studies, the clinicopathological mechanisms may be entirely different for GBC.

Blood glucose levels were shown to be associated with incidence of cancer overall and in several specific sites like the colon, pancreas, liver, and endometrium in previous studies [11,28,47 48]. Studies reporting specifically a link between GBC and blood glucose levels are almost inexistent [28]. The association between glucose and cancer risk in our study remained after adjustment for major putative confounders like BMI, smoking and age, indicating a possible causal link. However, biological mechanisms in the association between blood glucose and cancer are poorly understood. A large case-control study by associated with high blood pressure, none of these studies identified blood pressure as risk for GBC.

A case-control study on serum lipids and biliary tract cancers including gallbladder cancer showed that compared to controls, cases had significantly higher mean levels of serum triglyceride (STG) [15]. However, our study, based on prospective data analyses as well as other similar cohort studies did not confirm this finding [48,52]. In the study by Andreotti et al serum measurement took place shortly after cancer diagnosis. In this constellation one cannot rule out a possible reverse causation due to disease effect [53].

In conclusion, our study showed that increasing BMI and blood glucose levels are possible risk factors for GBC. Obesity was seen to pose a greater risk among women in the premenopausal age. Beyond the individual factors, the results of our study show that the metabolic syndrome as an entity presents a risk constellation for the occurrence of gallbladder cancer. Considering the rise in temporal trend of BMI and blood glucose levels [48,54], we would anticipate that the incidence ofShebl et al indicated that although diabetes could be a risk factor for gallstone formation, the association between diabetes and GBC can be explained only

partly by the positive association between diabetes and gallstones [14].

The inverse association we observed between total cholesterol and GBC in women may be largely due to preclinical effects of the cancer on total serum cholesterol [49]. A lag-time sub-analysis excluding 3 years of follow up after baseline measurement, rendered the association non-significant although the direction of association persisted. This was also shown in another recently published Me-Can study on total serum cholesterol and cancer [50].

Studies on the association between blood pressure and GBC incidence are scarce [48,51]. Although it was shown that several cancer sites might be significantly GBC might also increase.

Supporting Information

File S1 Table S1. Risk of primary gallbladder cancer (n = 91) in relation to quintiles of metabolic factors in men (n = 288,070). Table S2. Risk of primary gallbladder cancer (n = 91) in relation to quintiles of metabolic factors in women (n = 287,320). Table S3. Risk of primary gallbladder cancer (n = 184) by unit increment of z-scores of the metabolic factors and of the MetS score in men (n = 288,070) and in women (n = 287,320). Table S4. Risk of primary gallbladder cancer (n = 184) by WHO categories of metabolic factors in men (n = 288,070) and in women (n = 287,320).
(DOC)

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Author Contributions

Conceived and designed the experiments: WB ME TB CH BL GN AE TS SS JM RS ST HC GH HJ PS HU. Analyzed the data: WB ME SS TS CH HJ HU. Contributed reagents/materials/analysis tools: TB JM HC GH. Wrote the paper: WB ME TB CH BL GN AE TS SS JM RS ST HC GH HJ PS HU.

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CORRECTION:

This corrects table 2 (RR and 95% CI under model 2 for Triglyceride) of paper V

(Borena W, Edlinger M, Bjørge T, Ha¨ggstro¨m C, Lindkvist B, et al. (2014) A Prospective Study on Metabolic Risk Factors and Gallbladder Cancer in theMetabolic Syndrome and Cancer (Me-Can) Collaborative Study. PLoS ONE 9(2): e89368. doi:10.1371/journal.pone.0089368).

Table 2. Risk of primary gallbladder cancer (n=184) in relation to quintiles of metabolic factors (n=575,390)

Exposure	Quintile level	Mean (SD)	n	Model 1 [1] RR	95% CI	Model 2 [2] RR	95% CI
BMI (kg/m^2)	1	20.7 (1.3)	20	1.00			
	2	23.0 (0.8)	26	1.12	0.58, 2.19		
	3	24.7 (0.8)	38	1.49	0.80, 2.76		
	4	26.8 (0.9)	47	1.70	0.93, 3.09		
	5	31.3 (2.6)	53	1.94	1.08, 3.51		
				P_{trend} = 0.08			
Mid-BP [3] (mmHg)	1	88.2 (4.9)	20	1.00		1.00	
	2	96.9 (2.4)	27	1.37	0.48, 4.01	1.27	0.44, 3.74
	3	102.7 (2.3)	41	2.11	0.77, 5.75	1.86	0.68, 5.04
	4	109.8 (2.9)	35	1.02	0.36, 2.86	0.82	0.29, 2.32
	5	124.5 (9.5)	60	1.81	0.68, 4.81	1.25	0.45, 3.45
				P_{trend} = 0.47		P_{trend} = 0.92	
Glucose (mmol/l)	1	4.2 (0.5)	31	1.00		1.00	
	2	4.8 (0.3)	34	2.51	0.47, 13.5	2.32	0.43, 12.7
	3	5.1 (0.3)	28	1.18	0.20, 7.11	1.07	0.18, 6.33
	4	5.5 (0.4)	38	3.14	0.60, 16.5	2.64	0.49, 13.9
	5	6.8 (2.0)	53	7.52	1.56, 36.1	5.38	1.11, 26.5
				P_{trend} = 0.01		P_{trend} = 0.04	
Cholesterol (mmol/l)	1	4.2 (0.5)	27	1.00		1.00	
	2	5.0 (0.3)	37	1.14	0.53, 2.47	1.11	0.52, 2.38
	3	5.6 (0.3)	34	0.74	0.33, 1.61	0.70	0.32, 1.53
	4	6.2 (0.3)	40	0.71	0.35, 1.53	0.66	0.31, 1.42
	5	7.4 (0.8)	46	0.67	0.32, 1.46	0.62	0.29, 1.32
				P_{trend} = 0.14		P_{trend} = 0.08	
Triglycerides [4] (mmol/l)	1	0.7 (0.2)	22	1.00		1.00	
	2	1.0 (0.2)	30	1.48	0.45, 4.88	1.38	0.38, 2.00
	3	1.3 (0.3)	34	1.40	0.44, 4.49	1.20	0.37, 3.84
	4	1.9 (0.4)	46	2.50	0.84, 7.61	1.94	0.64, 5.94
	5	3.1 (1.7)	48	2.06	0.67, 6.28	1.38	0.44, 4.34
				P_{trend} = 0.12		P_{trend} = 0.50	

[1] RRs were estimated from Cox PH regression models with attained age as time scale after excluding the first year after baseline measurement; RRs are adjusted for smoking status and age at baseline, and stratified by cohort, sex and categories of birth year; RRs are corrected for regression dilution bias by use of the regression dilution ratio; conversion into uncorrected RR = exp (log(RR)*RDR); BMI RDR=0.90, mid-BP RDR=0.54, glucose RDR=0.29, cholesterol RDR=0.66, triglycerides RDR=0.47

[2] Additionally adjusted for quintile levels of BMI (except for BMI)

[3] Mid-BP is (systolic BP + diastolic BP) / 2; value missing for 1 GBC case

[4] Value missing for 4 GBC cases

Abbreviations: BMI=body mass index; BP=blood pressure; GBC=gallbladder cancer; RDR=regression dilution ratio; RR=relative risk; SD=standard deviation

This corrects the sub-section „Comparison with literature" of the "Discussion" section of paper V

(Borena W, Edlinger M, Bjørge T, Ha¨ggstro¨m C, Lindkvist B, et al. (2014) A Prospective Study on Metabolic Risk Factors and Gallbladder Cancer in the Metabolic Syndrome and Cancer (Me-Can) Collaborative Study. PLoS ONE 9(2): e89368. doi:10.1371/journal.pone.0089368)

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Although several independent mechanisms are depicted to underpin the association between obesity and cancer, the mechanisms that link obesity with gallbladder cancer risk are unclear (34-36). Many studies have identified obesity as a risk to be more pronounced in women, and suggested a possible role of sex hormones (mainly oestrogen) in the pathogenesis of GBC (8, 13, 29, 36-41). There are even indications that sex hormone receptors exist on the tumour tissue (42). Our

results, that increasing BMI poses greater risk mainly in younger women of premenopausal age group, might be supportive of this mechanism. This finding is supported by another cohort study in Norway (13).

In tumours that depend on oestrogen for their growth, like breast and endometrial cancers, obesity is shown to be of greater risk in postmenopausal women (43-46). In our study, however, the risk for GBC was higher for younger women below the age of 50. This observation might be due to chance, as we did not find a significant interaction between age and BMI. However, if confirmed in further studies, the clinicopathological mechanisms may be entirely different for GBC.

Blood glucose levels were shown to be associated with incidence of cancer overall and in several specific sites like the colon, pancreas, liver, and endometrium in previous studies (11, 28, 47-48). Studies reporting specifically a link between GBC and blood glucose levels are almost inexistent (28). The association between glucose and cancer risk in our study remained after adjustment for major putative confounders like BMI, smoking and age, indicating a possible causal link. However, biological mechanisms in the association between blood glucose and cancer are poorly understood. A large case-control study by Shebl et al indicated that although diabetes could be a risk factor for gallstone formation, the association between diabetes and GBC can be explained only partly by the positive association between diabetes and gallstones (14).

The inverse association we observed between total cholesterol and GBC in women may be largely due to preclinical effects of the cancer on total serum cholesterol (49). A lag-time sub-analysis excluding 3 years of follow up after baseline measurement, rendered the association non-significant although the direction of association persisted. This was also shown in another recently published Me-Can study on total serum cholesterol and cancer (50).

Studies on the association between blood pressure and GBC incidence are scarce (48, 51). Although it was shown that several cancer sites might be significantly associated with high blood pressure, none of these studies identified blood pressure as risk for GBC.

A case-control study on serum lipids and biliary tract cancers including gallbladder cancer showed that compared to controls, cases had significantly higher mean levels of serum triglyceride (STG) (15). However, our study, based on prospective data analyses as well as other similar cohort studies did not confirm this finding (48, 52). In the study by Andreotti et al serum measurement took place shortly after cancer diagnosis. In this constellation one cannot rule out a possible reverse causation due to disease effect (53).

In conclusion, our study showed that increasing BMI and blood glucose levels are possible risk factors for GBC. Obesity was seen to pose a greater risk among women in the premenopausal age. Beyond the individual factors, the results of our study show that the metabolic syndrome as an entity presents a risk constellation for the occurrence of gallbladder cancer. Considering the rise in temporal trend of BMI and blood glucose levels (48, 54), we would anticipate that the incidence of GBC might also increase.