Uterine fibroids and cardiovascular risk

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ABSTRACT

Study question:
Are uterine fibroids associated with increased cardiovascular risk?

Summary answer:
This study reports an association between increased serum lipids and metabolic syndrome with an increased risk of uterine fibroids.

What is known already:
Uterine fibroids are the most common tumour in females. Recent studies suggest similarities in biological disease mechanisms and risk factors for fibroids and atherosclerosis. Similar risk factors have been associated with both conditions: obesity, hypertension, and abnormal serum lipids. These findings are awaiting confirmation that a population based follow-up study could offer with extensive health examination data collection linked with a national hospital discharge register.

Study design, size, duration:
The Northern Finland Birth Cohort (NFBC1966) is a population-based long-term follow-up study including all children with estimated date of delivery in 1966 in the Northern Finland area. The data were collected from national registries, postal questionnaires and clinical health examinations. The study population for this study comprised all females included in the NFBC 1966 that underwent an extensive clinical health examination at age 46 years (n=3,635), comprised the study population for this study.

Participants/materials, setting, methods:
All females included in the NFBC1966 who were alive and traceable (n=5,118) were invited for the 46-year follow-up study; 3,268 (63.9%) responded, returned the postal questionnaire and attended the clinical examination. Uterine fibroid cases were identified through the national hospital discharge register that has data on disease diagnoses based on WHO ICD-codes. Uterine fibroid codes, ICD-9: 218 and ICD-10: D25 were used for case identification. Self-reported fibroid cases were identified through the postal questionnaire.

Main results and the role of chance:
A total of 729 fibroid cases were identified, including 293 based on hospital discharge registries. With adjustment for BMI, parity, education, and current use of exogenous hormones the risk of prevalent fibroids rose significantly for every 1 mmol/l increase in LDL (OR=1.13, 95% CI 1.02 to 1.26 for all cases) and triglycerides (OR=1.27, 95% CI 1.09 to 1.49 for all cases). Metabolic syndrome associated with hospital discharge-based fibroid diagnosis (OR= 1.48, 95% CI 1.09 to 2.01). Additionally every 1 unit increase in waist-hip ratio associated with fibroids (OR=1.32, 95% CI 1.10 to 1.57).

Limitations, reasons for caution:
The case ascertainment may present some limitations. There was likely an under-identification of cases and misclassification of some cases as controls; this would have diluted the effects of reported associations. The data analysed were cross-sectional and therefore cause and effect for the associations observed cannot be distinguished.

Wider implications of the findings:
Increased serum lipids and metabolic syndrome are associated with increased risk of uterine fibroids. Along with central obesity these findings add to an increased risk for cardiovascular disease among women with fibroids. These observations may suggest that there are shared predisposing factors underlying both uterine fibroids and adverse metabolic and cardiac disease risk, or that metabolic factors have a role in biological mechanisms underlying fibroid development.
Study funding/competing interests:

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Keywords: uterine fibroids, epidemiology, population based birth cohort studies, cardiovascular risk, lipid metabolism, glucose metabolism
Introduction

Uterine fibroids are the most common tumour in females with incidence of nearly 70% by age 50 years (Baird, et al., 2003). Fibroids decrease the quality of life by causing significant morbidity for the women. The related symptoms are heavy and prolonged menstrual bleeding, anaemia secondary to bleeding, pelvic pain and pressure and reduced fertility (Stewart, 2001). Current treatment options for uterine fibroids are limited to hormonal treatments, surgery, and latest the high intensity focused ultrasound (HIFU). All therapies, however, associate with substantial side effects and risks. The mainstream medications, based on the NICE (2013) Fibroids guidelines (levonorgestrel, progestogen, combined oral contraceptives, GnRH, ulipristal acetate, tranexamic acid and NSAIDs) focus on easing the symptoms rather than targeting the specific molecular disease mechanisms. The significant surgical need for fibroid treatment is well reflected by the fact that uterine fibroids are the primary indication for hysterectomy (Brummer, et al., 2009, Farquhar and Steiner, 2002). Given all this, uterine fibroids cause significant financial burden for the society (Soliman, et al., 2015) with an annual cost of 52.7 million euros in UK (Fernandez, et al., 2009) alone. The field of uterine fibroid biology has achieved crucial breakthrough discoveries through next-generation genetic studies in the very recent past by the discovery of mutations in mediator complex subunit 12 (MED12), which is a subunit of the mediator complex that regulates global and gene-specific transcription. The mutation has been recognised with a frequency of 70% of uterine fibroids (Makinen, et al., 2011). Yet the fundamental fibroid development mechanisms are still to be revealed.

Recent studies have indicated an association between uterine fibroids and several cardiovascular disease (CVD) risk factors such as hypertension, obesity, abnormal serum lipids and carotid intima-media (CIM) thickness (Aksoy, et al., 2014, Boynton-Jarrett, et al., 2005, He, et al., 2013, Luoto, 2002, Luoto, et al., 2001, Sadlonova, et al., 2008, Silver, et al., 2005, Summers, et al., 1971, Templeman, et al., 2009). The largest cohort study on fibroids, the Nurses’ Health Study II (NHS II) provides evidence for association of both hypertension (Boynton-Jarrett, Rich-Edwards, Malspeis, Missmer and Wright, 2005) and obesity (Marshall, et al., 1998) with increased risk of fibroids. Other studies have had limitations regarding case definition, study population, sample
size, or reproducibility. A comprehensively assessed study on metabolic and cardiovascular risk profiles and their association to uterine fibroids would bring further understanding to this study field. The Northern Finland Birth Cohort 1966 (NFBC1966) is a prospective population-based study comprising more than 12,000 participants with follow-up from birth to, currently, age 46 years. The present study utilizes extensive health examination data collected from women at age 46 years linked with national hospital discharge registers to evaluate the association between uterine fibroids and several CVD risk factors and metabolic factors. Measures for body mass index (BMI), waist and hip circumferences, body composition, oral glucose tolerance, International Diabetes Federation (IDF)-defined metabolic syndrome, serum lipids, sex hormones and high sensitivity C-reactive protein (hs-CRP), brachial blood pressure, and fatty liver index (FLI) were assessed. In addition, the present study investigated patient-specific CVD risk assessment scores (Framingham Risk Score and SCORE) to evaluate the metabolic risk for future cardiovascular events in women with uterine fibroids.
Materials and Methods

Study population

The study population derives from the prospective Northern Finland Birth Cohort 1966 (NFBC1966), comprising 96.3% of all estimated births in the two northernmost provinces of Finland (Oulu and Lapland) during the year 1966. NFBC1966 originally included 12,068 mothers who gave birth to 12,231 live born children (173 stillbirths), of whom 5,889 were females, all Caucasian. Data collection begun at gestational week 24 and so far data has been collected at ages 1, 14, 31 and 46 years. The women who were alive and whose contact information was traceable (n = 5,118) received an invitation for the 46-year follow-up study. In addition to a postal questionnaire, women were invited to a clinical health examination. 3,733 (72.9%) responded and returned the questionnaire and 3,268 (63.9%) attended the clinical examination and gave blood samples.

Ethics Statement

The Ethical Committee of the Northern Ostrobothnia Hospital District approved the study, which followed the principles of the Declaration of Helsinki. The participants took part on a voluntary basis and signed their informed consent. The data were handled on a group level only, the personal information being replaced by identification codes.

Ascertainment of uterine fibroid

Uterine fibroid cases were identified in the cohort through national outpatient and inpatient hospital discharge register that has data on disease diagnoses identified by WHO ICD disease codes. The national hospital discharge register includes ICD-codes and dates for each hospital visit, and since year 1968 this data has been regularly collected for the cohort population database. In the Finnish Healthcare system ICD-codes are used primarily for clinical diagnoses purposes, and secondly for municipal billing purposes. The ICD-codes are set by the clinical doctor who is in charge of discharging the patient, and the codes are chosen based on their clinical relevance for each hospital visit. Thus the disease diagnoses data is considered accurate and reliable. Uterine fibroid codes, ICD-9: 218 and ICD-10: D25 were used for case identification. Earlier used ICD-8 codes have been converted to ICD-9 codes and thus were included. This case ascertainment method identifies those fibroid patients who had required...
referral from primary health care or private sector to clinical review in specialty care; usually for consideration of surgical treatment or medical treatment after first and/or second line treatments have been unsuccessful. Henceforth those cases have been identified to whom fibroid diagnosis was of clinical significance with substantial symptoms. The fibroid diagnosis had been confirmed by a clinician, based either on clinical examination, imaging (ultrasonography, or other such as MRI or CT) or surgery. Additionally, self-reported fibroid cases were identified through the postal questionnaire collected at age 46 years with a question “Have you been diagnosed with uterine fibroids? If yes, at what age? If yes, was the diagnosis confirmed by gynaecological examination / ultrasonography / surgical operation (laparoscopy or laparotomy)?” Only cases with self-reported confirmation by either ultrasonography or surgical operation were recognised. A validation study was performed to assess reliability of the self-identified fibroids were confirmed by ICD-codes. Finally, the control group was formed of the rest of the cohort population (Figure 1).

Anthropometric measurements
All clinical health examinations took place and all measurements were taken at age 46 years. Body weight was measured with digital scale, which was calibrated regularly. Height was measured twice (mean of the two measurements was used) by using standard and calibrated stadiometer. Body mass index (BMI) was calculated as the ratio of weight and height squared. Waist and hip circumferences were measured twice (mean of the two measurements was used) and the waist-hip ratio (WHR) was assessed as the ratio between circumferences of the waist (at the level midway between lowest rib margin and the iliac crest) and the hip (at the widest trochanters). Body fat mass, fat percentage, muscle mass and visceral fat area were measured by InBody 720 bioelectrical impedance analyser (Biospace Co., Ltd., Seoul, Korea). All measurements were done after overnight (12h) fasting period.

Cardiovascular measurements
Systolic and diastolic blood pressure was measured three times with 1 min interval after 15 min of rest on the right arm of the seated participants using an automated oscillometric blood pressure device and appropriately sized cuff (Omron Digital...
Automatic Blood Pressure Monitor Model M10-IT). Finally, the mean of two lowest systolic values and their diastolic values was used in the analyses.

Oral glucose tolerance test and diabetes

A two-hour oral glucose tolerance test (OGTT) was performed after overnight (12 hour) fasting period. Exclusion criteria were medication for diabetes or just before test measured capillary blood glucose level >8.0 mmol/l. Both serum insulin and plasma glucose were measured at baseline and 30, 60 and 120 minutes after 75g glucose intake.

Glucose tolerance status was classified according to World Health Organization (WHO) criteria: 1) normal glucose tolerance (NGT) was defined as having fasting plasma glucose (FPG) level <6.1 mmol/l and 2-hour glucose level <7.8 mmol/l, 2) impaired fasting glucose (IFG) as having FPG level 6.1-6.9 mmol/l and 2-hour glucose level <7.8 mmol/l, 3) impaired glucose tolerance (IGT) as having FPG level <7.0 mmol/l and 2-hour glucose level 7.8-11.0 mmol/l, and 4) screen detected diabetes mellitus (sCDM) as having FPG level ≥7.0 mmol/l and/or 2-hour glucose level ≥11.1 mmol/l. Fasting glucose and insulin values were used to calculate fasting indices: HOMA-IR index (Homeostasis Model Assessment – insulin resistance) (FPG x FSI / 22.5), HOMA2-β index (Homeostasis Model Assessment – beta-cell function) ((20 x FSI) / (FPG – 3.5) x 100)) and QUICKI index (Quantitative Insulin Sensitivity Check Index) (1 / (log FBG + log FPI). OGTT glucose and insulin values were used to calculate insulin and glucose area under curve (glucose-AUC and insulin-AUC), several indices for insulin sensitivity: Matsuda index (ISI) (10 000 x ((FPG x FSI) x ((FPG + 30min PG + 60min PG + 120min PG) / 4) x ((FSI + 30min SI + 60min SI + 120min SI) / 4))), Belfiore index (2/((((0.5 * FPG) + 60min mean PG + (0.5 * 120min PG) * (((0.5 * FPG) + 60min PI + (0.5 * 120min PI)) / 638) + 1)), and Gutt index (75000 + (FPG – 120min PG * 0.19 * weight [kg] / 120) / ((FPG + 120min PG / 2) / LOG10 ((FPI + 120min PI) / 2)) (Belfiore, et al., 1998, Gutt, et al., 2000, Katz, et al., 2000, Matsuda and DeFronzo, 1999, Matthews, et al., 1985). Previously known diabetes (prDM) was defined according to self-reported diagnoses and medications, hospital outpatient and inpatient registers and medication registers from Social Insurance Institution of Finland.

Other biochemical measurements
Blood samples were taken after an overnight fasting period, centrifuged immediately and stored firstly at −20°C and later at −80°C. All blood samples were analysed in the laboratory of the University Hospital of Oulu according to a standardized protocol. Serum total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), and triglycerides were determined using an enzymatic assay method (Advia 1800; Siemens Healthcare Diagnostics Inc., Tarrytown, Ny, USA). Serum samples for testosterone (T) were analysed using Agilent triple quadrupole 6410 LC/MS equipped with electrospray ionisation source operating with positive-ion mode (Agilent Technologies, Wilmington, DE, USA). Multiple reaction monitoring was used to quantify testosterone by d3-testosterone with the following transitions: m/z 289.2 to 97 and 289.2 to 109 for testosterone and 292.2 to 97 and 292.2 to 109 for d3-testosterone. The intra-assay CVs of the method were 5.3%, 1.6 % and 1.2 % for testosterone at 0.6, 6.6 and 27.7 nmol/l, respectively. The interassay CVs were 5.3%, 4.2% and 1.0% for the respective concentrations. Serum sex hormone-binding globulin (SHBG) was analyzed by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare Diagnostica Inc., Llanberis, UK). High sensitivity C-reactive protein (hs-CRP) was analyzed by an immune nephelometric assay (BN ProSpec, Siemens Healthcare Diagnostics Inc., Newark, DE, USA).

**CVD risk assessment scoring**

Two CVD risk assessment tools were used, Framingham Risk Score (D'Agostino, et al., 2008) and SCORE (Conroy, et al., 2003). The Framingham Risk Score estimates a 10-year risk of developing coronary heart disease, cerebrovascular events, peripheral artery disease or heart failure. It bases its risk-percentage result on the following factors: gender, age, smoking, total cholesterol, HDL-cholesterol, systolic blood pressure, requiring treatment for raised blood pressure, and diabetes. As a result the Framingham risk assessment tool reports points ranging from ≤2 to ≥21 that refer to a risk percentage ranging from <1% to ≥30% (D’Agostino, Vasan, Pencina, Wolf, Cobain, Massaro and Kannel, 2008). The SCORE estimates a 10-year risk of fatal cardiovascular disease on the basis of gender, age, smoking, total cholesterol and systolic blood pressure. It results risk percentages ranging from <1% to ≥15% (Conroy, Pyorala, Fitzgerald, Sans, Menotti, De Backer, De Bacquer, Ducimetiere, Jousilahti,
Metabolic syndrome
Metabolic syndrome was assessed according to the International Diabetes Federation (IDF) Worldwide Definition (Alberti, et al., 2006), which is a unified working diagnostic tool for the metabolic syndrome. The tool results with yes or no for metabolic syndrome based on central obesity measured by waist circumference (≥80cm) and any two of the following: raised triglycerides (≥1.7mmol/l or specific treatment for this lipid abnormality), reduced HDL (<1.29mmol/l or specific treatment for this lipid abnormality), raised blood pressure (systolic ≥130mmHg or diastolic ≥85mmHg or treatment of previously diagnosed hypertension), raised fasting plasma glucose (≥5.6mmol/l or previously diagnosed type 2 diabetes).

Fatty liver index
Fatty liver disease is strongly associated to obesity and it can be predicted using a fatty liver index (FLI) algorithm, which is based on BMI, central obesity measured by waist circumference, triglyceride and gamma-glutamyl-transferase (GGT) levels (Bedogni, et al., 2006). FLI is calculated as: FLI = (e^{0.953*\log_e(triglycerides) + 0.139*BMI + 0.718*\log_e(ggt) + 0.053*waist circumference - 15.745}) / (1 + e^{0.953*\log_e(triglycerides) + 0.139*BMI + 0.718*\log_e(ggt) + 0.053*waist circumference - 15.745}) * 100. FLI varies between 0 and 100, with cut offs at 30 and 60; score <30 rules out fatty liver and a score ≥60 is considered a strong predictor for fatty liver.

Confounders
Data on parity was obtained from the postal questionnaire and national birth register. Exogenous hormone use, both lifetime and current, and self-reported polycystic ovary syndrome (PCOS) was obtained from the postal questionnaire. Menopausal status was determined by a question on climacteric symptoms on the postal questionnaire. Women reported their lifetime education level, which was categorised as basic, secondary, or tertiary. Socioeconomic status was again determined by questions on the postal questionnaire and categorised as entrepreneur, higher administrative, lower administrative, blue-collar worker, or other. Women reported their leisure-time
physical activity level in the questionnaire before health examinations. The four
categories were named inactive, lightly active, active, or very active. Smoking history
and present status was inquired and results reported as non-smoker, former smoker
more than 6 months ago, former smoker less than 6 months ago, or current smoker.
Women reported their alcohol consumption and the four categories were named non-
user, light user, moderate user, or heavy user.

Statistical methods

Distributions of continuous variables were expressed as mean ± standard deviation
(SD), and categorical variables as numbers and percentage of proportions. The χ²-test
and Mann-Whitney U test were used to study associations between uterine fibroid cases
and controls with cardiovascular risk factors.

Logistic regression analysis was used to examine associations between uterine fibroid
cases and controls with known cardiovascular risk factors. The analysis was first
performed for all fibroid cases and then for ICD-code identified cases. BMI, parity,
education and current use exogenous hormones were used as potential confounding
factors in the analyses (Table I). P-value <0.05 was considered statistically significant.
Statistical analyses were conducted using the free software package R 3.1.0.
Results

We identified 729 uterine fibroid cases in the NFBC1966 by year 2012 for this study. Of these cases 293 were identified through WHO ICD disease codes for uterine fibroids. The rest of the cohort population formed the control group (n = 2,906) (Figure 1).

Figure 2 presents the overall ICD-code based uterine fibroid incidence in the cohort. This includes all women who participated in either the postal questionnaires or the clinical examinations. Cumulative incidence was 7.7%. The number of newly detected cases starts to increase after age 41 years, and by age 46 years there were a total of 395 ICD-code identified fibroid cases in the cohort. The mean age of fibroid diagnosis was 37.3 years (median 40.0, SD 7.1, range 13-47), and for the ICD-code confirmed fibroid cases 40.2 years (median 42 years, SD 5.3, range 23-46).

Confounders

Women with uterine fibroids had significantly lower parity than women without fibroids (mean 1.8 SD 1.7 vs. 2.2 SD 1.8, P<0.001) (Table I). There were significant differences in body size among women with and without uterine fibroids. Women with fibroids were more frequently overweight or obese (35.4% and 21.8% vs. 31.6% and 21.4%, P=0.04). There were no differences in lifetime use exogenous hormones, but current use differed in hormonal replacement therapy for all fibroids (3.8% vs. 2.4%, P=0.04). There were no differences in polycystic ovary syndrome or menopausal status defined by experienced climacteric symptoms. Again, there were no significant differences in regards of cigarette smoking, alcohol use, or physical activity.

Women with fibroids had a lower lifetime education level (basic 3.8% and tertiary 39.8% vs. 2.0% and 41.6%, P=0.012), but they did not differ in their socioeconomic status when compared to women without fibroids (Table I). These results defined the adjustment models for parity, education level, BMI, and current use exogenous hormones as applied in the analyses.

Anthropometrics

With logistic regression analysis, the odds ratio of uterine fibroid diagnosis (all cases, and WHO ICD-code identified cases) according to several known cardiovascular disease risk factors was estimated. With adjustment for parity, education, BMI, and current use exogenous hormones the risk of prevalent fibroids rose significantly for
every 1cm increase in waist circumference (OR=1.02, 95%CI 1.00 to 1.04) (Table II).

Also every unit increase in WHR associated with fibroids (OR=1.32, 95%CI 1.10 to 1.57) (Table II). However, increase in body composition: fat percentage, fat mass, skeletal muscle mass, visceral fat area, did not show association to fibroids (Table II).

The results for ICD-code confirmed cases were congruent (Table II).

**Glucose metabolism**

The 2-hour OGTT results suggest a positive association between glucose metabolism and uterine fibroid risk. This is shown in insulin levels at 60 minutes when adjusting for parity and education (All uterine fibroid cases: OR=1.02 95%CI 1.00 to 1.04) and in glucose levels at 30 minutes (ICD-code confirmed cases: OR=1.13 95%CI 1.03 to 1.24) (Table III). Interestingly, glucose tolerance results meeting the criteria of IFG showed a significant association among the ICD-code confirmed cases only in the fully adjusted model (OR=1.81 95%CI 0.98 to 3.14) (Table III).

**Lipid metabolism**

Serum lipids associated with uterine fibroids independent of parity, education, BMI, and current use exogenous hormones. For every 1mmol/l increase in LDL and triglycerides the risk of prevalent fibroids rose significantly (OR=1.13 95%CI 1.02 to 1.26 and OR=1.27 95%CI 1.09 to 1.49) (Table IV). The associations were stronger for hospital discharge defined fibroid cases (OR=1.22 95%CI 1.05 to 1.42 and OR=1.37 95%CI 1.11 to 1.68). Additionally for these cases, every 1mmol/l increase in total cholesterol associated with fibroids (OR=1.21 95%CI 1.05 to 1.41). These associations were not altered when the model was additionally adjusted for polycystic ovary syndrome.

**Blood pressure, metabolic syndrome and cardiovascular risk scores**

Blood pressure measured at age 46 years did not show association with uterine fibroid risk (Table IV). IDF defined metabolic syndrome associated with hospital discharge based fibroid diagnosis significantly, independent of parity, education, BMI, and current use exogenous hormones (OR=1.48 95%CI 1.09 to 2.01). The CVD risk assessment scoring was performed by using two widely used tools; Framingham CVD risk score and SCORE. The analysis did not show significant association with fibroids according to either CVD risk assessment scores, in any of the adjustment models.
Liver function and chronic inflammation

Fatty liver index (FLI) was not associated with uterine fibroids in this analysis. When adjusting for parity and education, hs-CRP 1-3 mg/l associated with hospital discharge based fibroid diagnosis (OR=1.35 95%CI 1.01 to 1.80) (Table IV), but this association became non-significant after adjusting additionally for BMI and current use exogenous hormones (Table IV).

Sex hormones

With the full adjustment model, no association was observed for SHBG (Table IV). However, every 1 nmol/l increase in serum total testosterone associated with ICD-code confirmed fibroid cases (OR=0.60 95%CI 0.40 to 0.89) (Table IV).
Discussion

In this population-based birth cohort study we report unfavourable alterations in several well-documented cardiovascular disease risk factors in women diagnosed with uterine fibroids. At the age of 46 years, increased serum total cholesterol, LDL and triglyceride levels were associated with increased risk of fibroids. The odds ratios were higher among women with hospital discharge based WHO ICD-code for fibroid diagnosis, suggesting stronger associations of the tested risk factors among women with more severe fibroid related symptoms and thus large or multiple fibroids. Additionally, impaired glucose tolerance and metabolic syndrome were associated with fibroid risk.

Strengths and weaknesses of the study

Our analyses were conducted in a large population based cohort with accurate data on medical diagnoses at specialty care, vast clinical examinations and extensive questionnaires. The cohort database has precise data on body size and an extensive range of assayed metabolic biochemical markers. The strength of our study is that we were able to analyze all CVD risk factors simultaneously in the same study population, in the same time period.

The case ascertainment may present some limitations. There was likely an under-ascertainment of cases and misclassification of some cases as controls due to the fact that uterine fibroids may present as asymptomatic and thus remain undiagnosed. This would have diluted the effects of reported associations. The incidence of fibroids in this study was 20.1% (729/3635) when considering all cases, and 8.1% (293/3635) of ICD-code identified cases, whereas the overall ICD-code based incidence in the cohort, when including all women despite their participation to the clinical examinations, was 7.7%. Indeed there is a discrepancy when comparing this figure to the reported cumulative incidence among White women in their late 40s by Baird 2003; ~70% in ultrasound screened study population and 35% in clinically relevant cases. There are no comparable figures for Finnish population, but an ultrasound screening study presents a fibroid prevalence for Swedish women aged 33 to 40 years to be 7.8% (Borgfeldt and Andolf, 2000), which is more in proportion to our findings. Age of the cohort at the time of clinical examinations was not ideal for cardiovascular risk assessment, as age is the strongest risk factor for cardiovascular disease and...
the risk starts to rise significantly after the age of 60 years (Tuomilehto, 2004). The data analysed were cross-sectional and therefore cause and effect for the associations observed cannot be distinguished.

**Comparison to other studies**

The association between obese body size and uterine fibroids has been confirmed by many studies, although not all studies agree (Table V). In the previous studies body size has been determined by calculating BMI and the results are fairly consistent; data arising from the NHS II with 2,967 identified fibroid cases shows increased risk of fibroids with increasing adult BMI (Marshall, Spiegelman, Manson, Goldman, Barbieri,Stampfer, Willett and Hunter, 1998). Furthermore, central obesity as measured by WHR has been associated with increased risk of fibroids (Sato, et al., 1998, Wise, et al., 2005). Our study confirms the association, as increase in waist circumference and WHR was associated with an increased risk of fibroids. Other adiposity traits (visceral fat or gynecoid pattern fat accumulation), examined through body fat distribution using bioelectrical impedance analysis, did not show association.

Lipid metabolism in women with uterine fibroids has been analyzed in only few studies and the results are conflicting (Table V). All studies are case-control settings with small sample sizes. In the present study the lipid levels were assessed at the same age for all cohort participants showing a positive association between LDL and triglycerides and risk of fibroids. The unfavourable trend of serum lipid levels seems to be independently associated with fibroids, as when adjusting for parity, education and BMI the results remain statistically significant.

Published data on the association between glucose metabolism and uterine fibroids is limited. The association has been analysed using mainly self-reported diabetes diagnosis, but also with fasting glucose levels, fasting insulin levels and short insulin tolerance test (Table V). No clear association has been documented in these studies between changes in glucose metabolism and uterine fibroids. The present study analyzed the association with a vast set of glucose metabolism tests and indices, again at the same age for all cohort participants. Women with ICD-code confirmed fibroids showed association to impaired fasting glycemia (IFG), which refers to constant
elevation of fasting plasma glucose levels. It can progress to more severe forms of
glucose intolerance and further on to diabetes, and thus is considered as a pre-diabetic
state (Nichols, et al., 2007). Additionally, the ICD-code confirmed fibroid cases showed
association to IDF-defined metabolic syndrome, clustering the effects of several
metabolic traits to have association to fibroids, and infer of adverse cardiovascular
events, which is the main adverse outcome of metabolic syndrome (DeFronzo and
Abdul-Ghani, 2011, Mottilo, et al., 2010).

There is evidence that hypertension and uterine fibroids are associated. The relation has
been shown in many studies, but also suggestion of no association has been published
(Table V). However, the NHS II, which is the largest study on fibroids by to date, offers
strong evidence on the association and reports every 10 mmHg increase in diastolic
blood pressure increased the risk of fibroids by 8% among non-users and 10% among
users of antihypertensive medications (Boynton-Jarrett, Rich-Edwards, Malspeis,
Missmer and Wright, 2005). Our analyses of the NFBC1966 cohort did not support an
association between uterine fibroids and hypertension. One reason for this may be the
relatively young age of the cohort.

Potential mechanisms
First identifications of possible underlying atherosclerotic mechanisms in uterine
fibroid development arise from studies performed in the 1970s, when fibroid tissue and
atherosclerotic plaque were recognised to have similarities in growth behaviour as they
both become fibrotic and calcified (Moss and Benditt, 1975). Further suggestions on
atherosclerotic mechanisms arise from notifications of lipid accumulation in
myometrial smooth muscle cells in women with pregnancy related hypertension (Haust,
et al., 1977), vascular endothelial and myometrial smooth muscle cells seem to react
similarly to injury and promote monoclonal expansion of smooth muscle cells in the
uterine wall (Cramer, et al., 1995). Monoclonal theory of origin is another shared
similarity of these two phenomena (Andreassi, et al., 2000, Benditt and Benditt, 1973,

Obesity is associated with different grades of insulin resistance, which is a substantial
underlying key process in the development of cardio-metabolic disorders. Central
obesity in particular raises the risk for development of metabolic complications, with
mounting evidence that not only visceral adipose tissue, but also subcutaneous adipose
tissue has a significant impact on the process (Patel and Abate, 2013). In fact, fat distribution in obese premenopausal women is more often characterised with excess subcutaneous fat, but changes in menopause transition to visceral fat accumulation (Toth, et al., 2000). In the first phase of insulin resistance, hyperinsulinemia increases the hepatic synthesis and activity of insulin-like growth factors, such as IGF-I. Insulin resistance seems to play role in uterine fibroid development, as IGF-I may act to promote fibroid growth in an autocrine/paracrine fashion. IGF-I receptors are increased in fibroid tissue compared to myometrium (Chandrasekhar, et al., 1992), and the levels of IGF-I peptide, IGF-I mRNA and IGF-II mRNA are also elevated (Englund, et al., 2000, van der Ven, et al., 1994, Vollenhoven, et al., 1993). A recent study on experimental mouse model reported of induced insulin resistance and administration of oestrogen and progesterone hormones to promote uterine smooth muscle growth, where insulin resistance had an enhancing effect on the steroid hormone stimulation (Hou, et al., 2015). The authors suggest that this might imply of a possible effect of insulin resistance in the development of uterine fibroids. Again, an association study on fibroid tumor size across extended candidate chromosomal regions for uterine fibroids resulted with a sole significant variant in SORCS2 (sortrin-related VPS10 domain containing receptor 2) gene (Aissani, et al., 2015), which is also a strong candidate gene for circulating IGF-I and IGFBP-3 (Kaplan, et al., 2011).

Adding these observations together, it can be proposed that simultaneous and alike disease mechanisms occur in both myometrial smooth muscle and vascular endothelial cells, that would initially be provoked by unfavourable metabolic circumstances. Genes encoding mediator complex have been associated with both uterine fibroids and metabolic syndrome (Makinen, Mehine, Tolvanen, Kaasinen, Li, Lehtonen, Gentile, Yan, Enge, Taipale, Aavikko, Katainen, Virolainen, Bohling, Koski, Launonen, Sjoberg, Taipale, Vahteristo and Aaltonen, 2011, Schiano, et al., 2014). Exploring the biological pathways involving mediator subunits is one way of investigating the common biology between these traits.

**Conclusions**

Our study provides evidence for uterine fibroids and outcomes that are also associated with cardiovascular disease. According to this large birth cohort study, unfavourable lipid and glucose metabolism, and metabolic syndrome are associated with increased risk for uterine fibroid diagnosis. Along with central obesity these associating factors...
accumulate to increased risk for cardiovascular disease among women with uterine fibroids. In conclusion, the observed associations may suggest that there are shared predisposing factors underlying both uterine fibroids and adverse metabolic and cardiac disease risk, or that metabolic factors have a role in biological mechanisms underlying fibroid development. However, the causality cannot be determined by a cross-sectional study as ours, and therefore longitudinal prospective studies are needed to further investigate the underlying biological mechanisms in fibroid development.
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References


**Figure Legends**

**Figure 1** Participants of the uterine fibroid and cardiovascular risk NFBC1966 study.

**Figure 2** Uterine fibroid incidence in the NFBC1966. Cases were identified through the national hospital discharge register based on WHO ICD-codes for uterine fibroids (ICD-9: 218 and ICD-10: D25, ICD-8 converted to ICD-9).