Cardiovascular Risk Factors and Arterial Function After Gestational Diabetes Mellitus
Role of obesity and metabolic syndrome
TIINA VILMI-KERÄLÄ

Cardiovascular Risk Factors and Arterial Function After Gestational Diabetes Mellitus

Role of obesity and metabolic syndrome

ACADEMIC DISSERTATION
To be presented, with the permission of the Faculty Council of the Faculty of Medicine and Life Sciences of the University of Tampere, for public discussion in the auditorium of Finn-Medi 5, Biokatu 12, Tampere, on 24 August 2018, at 12 o’clock.

UNIVERSITY OF TAMPERE
TIINA VILMI-KERÄLÄ

Cardiovascular Risk Factors and Arterial Function After Gestational Diabetes Mellitus

Role of obesity and metabolic syndrome

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Tampere 2018
To my Family:

Johannes, Vilma, Elias and Niilo

Obstacles are those frightful things you see when you take your eyes off your goals.

~ Henry Ford
ABSTRACT

Gestational diabetes mellitus (GDM) is a common metabolic complication that affected 17.5% of pregnancies in Finland in 2016. Although glucose homeostasis most often normalizes after delivery, women with previous GDM have a sevenfold risk of type 2 diabetes mellitus (T2DM) in the future. Moreover, affected women are also at an increased risk of developing cardiovascular disease (CVD) or metabolic syndrome (MetS) later in life. MetS is an accumulation of disadvantageous health conditions, and although it is evidently associated with the risk of CVD, occasionally its utility in this regard has been questioned in general practice. Nevertheless, MetS is a growing issue and it is linked to many conditions unique to women’s health, including GDM.

With this background, the aim of this study was to examine (in a setting of two cohorts) whether or not women’s CVD risk, assessed by traditional as well as novel biomarkers and measures of arterial function, is already increased a few years after GDM. Additionally, another goal was to compute the effect of obesity on the results. Further, we wanted to study the utility of MetS diagnosis when estimating individualized CVD risk. For this, differences in arterial stiffness were determined between individually paired fertile women with and without MetS.

Altogether, 240 women were selected in the follow-up study of two cohorts, and all of the women had both delivered in Kanta-Häme Central Hospital during 2008–2011 and undergone a 75-g oral glucose tolerance test during the index pregnancy. In Studies I–III, a total of 120 women with a history of GDM during the index pregnancy were compared with 120 age-matched women with normal glucose metabolism during pregnancy by assessing MetS prevalence, glucose and lipid metabolism, variables of low-grade inflammation and values of arterial function. To evaluate the effect of obesity on the results, the whole study population was divided into four subgroups according to body mass index (BMI) and previous GDM. In this original study population including 240 participants, there were 27 women with MetS. In Study IV, twenty-seven women with MetS were compared with individually matched counterparts without the syndrome. In addition to previous GDM, the counterparts without MetS were matched according to age, and serum concentrations of both LDL-cholesterol (LDL-C) and...
total cholesterol (TC). Further, there was no significant difference in smoking history between the individually paired study groups.

In Studies I–III, when investigated on average 3.7 years after delivery, women with a history of GDM were found to have a 2.4-fold increased prevalence of MetS, and they were also more insulin resistant (as measured by using homeostasis model assessment of insulin resistance [HOMA-IR]) than those without previous GDM. Reflecting low-grade inflammation in the GDM cohort, serum concentrations of tissue inhibitor of metalloproteinase-1 (TIMP-1) were significantly upregulated after prior GDM. Moreover, women with previous GDM had higher values of pulse wave velocity (PWV), indicating that their arteries are less distensible than those in women with previous normoglycemic pregnancy. Most of the findings were more evident in obese participants; the influence of obesity frequently exceeded that of GDM. In Study IV, when arterial function was measured by three non-invasive methods, fertile women with MetS had increased arterial stiffness, a predictor of future CVD events, when compared with individually paired women without the syndrome. These results support the clinical use of MetS when revealing increased individual CVD risk, particularly among fertile-aged women.

Väitöskirjatutkimuksen tavoitteena on ollut selvittää, onko aiemmissa tutkimuksissa osoitettu raskausdiabetesen jälkeinen kohonnut sydän- ja verisuonitautiriski todettavissa herkällä määrityksellä jo muutama vuosi synnytyksen jälkeen. Lisäksi on pyritty tutkimaan lisääntyvän lihavuuden vaikutuksia tuloksiin. Tutkimuksessa analysoitiin myös MBO-diagnoosin käyttökelpoisuutta klinisessä työssä arvioitaessa yksilön sydän- ja verisuonitautiriskiä.


Osatöissä I–III keskimäärin 3,7 vuotta synnytyksen jälkeen tehdyissä seurantatutkimuksissa todettiin, että raskausdiabeetikoilla esiintyi 2,4-kertaisesti
This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I–IV).


The publications were adapted with the permission of the copyright owners.
<table>
<thead>
<tr>
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<th>Full Form</th>
</tr>
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<tr>
<td>ACOG</td>
<td>American Congress of Obstetricians and Gynecologists</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>ALAT</td>
<td>alanine transaminase</td>
</tr>
<tr>
<td>AlbCre</td>
<td>albumin to creatinine ratio</td>
</tr>
<tr>
<td>AMI</td>
<td>acute myocardial infarction</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>C1</td>
<td>large arterial compliance</td>
</tr>
<tr>
<td>C2</td>
<td>small arterial compliance</td>
</tr>
<tr>
<td>C&amp;C</td>
<td>Carpenter and Coustan</td>
</tr>
<tr>
<td>cBP</td>
<td>central blood pressure</td>
</tr>
<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CV</td>
<td>cardiovascular</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>D</td>
<td>distance</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>Dt</td>
<td>time delay/transit time</td>
</tr>
<tr>
<td>f</td>
<td>fasting</td>
</tr>
<tr>
<td>fP</td>
<td>fasting plasma</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
</tbody>
</table>
GCT  glucose challenge test
GDM  gestational diabetes mellitus
Gluc  glucose
h  hours
HAPO  Hyperglycemia and Adverse Pregnancy Outcome
HbA1c  glycosylated hemoglobin A1c
HDL  high-density lipoprotein
HDL-C  high-density lipoprotein cholesterol
HOMA-IR  homeostasis model assessment of insulin resistance
HR  hazard ratio
hsCRP  high sensitivity C-reactive protein
IADPSG  International Association of the Diabetes and Pregnancy Study Groups
IDF  International Diabetes Federation
i.e.  id est
IFG  impaired fasting glucose
IGT  impaired glucose tolerance
Insu  insulin
IR  insulin resistance
LDL  low-density lipoprotein
LDL-C  low-density lipoprotein cholesterol
MetS  metabolic syndrome
MMP  matrix metalloproteinase
NCEP  National Cholesterol Education Program
NDDG  National Diabetes Data Group
NO  nitric oxide
NPV  negative predictive value
NS  nonsignificant
OGTT  oral glucose tolerance test
OR  odds ratio
oxLDL  oxidized low-density lipoprotein
PCOS  polycystic ovary syndrome
PCSK9  proprotein convertase subtilisin/kexin type-9
PP  pulse pressure
PPV  positive predictive value
PWV  pulse wave velocity
QUICKI  quantitative insulin sensitivity check index
ROS  reactive oxygen species
RR  relative risk
SD  standard deviation
TC  total cholesterol
TG  triglyceride
TIMP  tissue inhibitor of metalloproteinase
T1DM  type 1 diabetes mellitus
T2DM  type 2 diabetes mellitus
VLDL  very low-density lipoprotein
WC  waist circumference
WHO  World Health Organization
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INTRODUCTION

Gestational diabetes mellitus (GDM) has long been defined as glucose intolerance with first recognition during pregnancy (American Diabetes Association. 2003). In recent decades, the prevalence of GDM has multiplied globally along with increasing rates of obesity, advancing maternal age and inactive lifestyles (Dabelea et al. 2005, Schmidt et al. 2012, Vuori & Gissler. 2014). In Finland, GDM complicated 17.5% of pregnancies in 2016 (Vuori & Gissler. 2017). In most cases, glucose intolerance normalizes after delivery (Järvelä et al. 2006, Kim et al. 2002, The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 1997), but women with a history of GDM have at least a sevenfold risk of developing type 2 diabetes (T2DM) in the future (Bellamy et al. 2009). Additionally, affected women are at a higher risk of developing cardiovascular disease (CVD) or metabolic syndrome (MetS) years after the pregnancy (Goueslard et al. 2016, Y. Xu et al. 2014).

Metabolic syndrome (MetS) is an accumulation of disadvantageous health conditions, including central obesity, elevated blood pressure, dyslipidemia and abnormal glucose tolerance, which altogether increase the risk of cardiovascular disease (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002). MetS is a growing issue and linked to many conditions unique to women’s health, including GDM. The prevalence of MetS is higher in women and it has rapidly increased in recent decades in parallel with growing obesity and sedentary lifestyles (E. L. Miller & Mitchell. 2006, Y. Xu et al. 2014). The central component of MetS is insulin resistance, which is associated with an enhanced inflammatory state and vascular endothelial dysfunction (Pickup. 2004). Although MetS is evidently associated with the risk of CVD, in general practice its utility in this regard has occasionally been questioned (Balkau et al. 2002, Bauduceau et al. 2007, Borch-Johnsen & Wareham. 2010, Kahn et al. 2005, Mente et al. 2010, Simmons et al. 2010, Woodward & Tunstall-Pedoe. 2009).

Atherosclerosis is a chronic process that is crucial for the development of CVD (Furie & Mitchell. 2012, Rocha & Libby. 2009). It begins with accumulation of lipoproteins, particularly low-density lipoprotein (LDL), into the arterial wall,
which are then subjected to oxidative modifications (Stocker & Keaney. 2004). Circulating oxidized LDL (oxLDL) seems to reflect the level of oxidative stress (Sigurdardottir et al. 2002), and increased amounts of circulating oxLDL are associated with the occurrence of coronary heart disease (Holvoet et al. 1998, Holvoet et al. 2001).

Besides elevated oxidative stress, inflammation is important in atherosclerosis (Feng et al. 2011, Stocker & Keaney. 2004), and it seems to be a predictor of women’s cardiovascular (CV) complications (Ridker et al. 2002). Elevated levels of high-sensitivity C-reactive protein (hsCRP) represent a significant risk factor of atherosclerosis (Karadeniz et al. 2015). The group of matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), have also been related to the formation of atherosclerosis and its progression in humans (Goncalves et al. 2009, Paim et al. 2013, Siasos et al. 2012). Further, arterial endothelial dysfunction is a major, early, and possibly reversible step in the atherosclerotic process (Berliner et al. 1995, Healy. 1990, Ross. 1993, Smith et al. 2004).

With this background, the present series of studies was aimed at exploring whether or not women’s CVD risk, assessed by traditional as well as novel biomarkers and values of arterial function, is already increased a few years after GDM. Another goal was to evaluate the effect of obesity on the results. Further, we wanted to study the utility of MetS diagnosis when estimating individual CVD risk. Therefore, differences in arterial stiffness were explored in individually paired fertile women with and without MetS.
2 REVIEW OF THE LITERATURE

2.1 Gestational Diabetes Mellitus

2.1.1 Definition and pathogenesis

In 1882, Matthews Duncan first reported that diabetes existing before pregnancy may have severe adverse effects on fetal and neonatal outcomes (Duncan. 1882). In the 1940s, it was recognized that women who developed diabetes years after pregnancy had suffered unusually high fetal and neonatal mortality (H. C. Miller. 1946). By the 1950s the term “gestational diabetes” was applied to a temporary hyperglycemic condition that influenced fetal outcomes unfavorably, which then was normalized after delivery (Carrington et al. 1957).

In 1965, the World Health Organization (WHO) Expert Committee on Diabetes Mellitus released the first guideline on diabetes, in which gestational diabetes mellitus (GDM) was defined as “hyperglycemia of diabetic levels occurring during pregnancy” (WHO. 1999). Consequently, GDM is a form of hyperglycemia (American Diabetes Association. 2003). For many years, it was defined as any degree of carbohydrate intolerance resulting in hyperglycemia with onset or first recognition during pregnancy (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 1997). According to Finnish Current Guidelines this still is the definition of GDM (Gestational diabetes. Current Care Guidelines. 2013). However, GDM can be diagnosed only when other types of diabetes are excluded. For example, nowadays couples are generally postponing parenthood across the developed countries (Schmidt et al. 2012). In Europe, the mean age of primiparous women has increased, being currently between 28 and 29 years (T. J. Matthews & Hamilton. 2014, Schmidt et al. 2012). With age, the prevalence of type 2 diabetes (T2DM) increases, and additionally, the ongoing epidemic of obesity has led to more T2DM in women of reproductive age. Therefore, there is an increased number of pregnant women with undiagnosed T2DM (Lawrence et al. 2008).
Normally, fasting and postprandial glucose concentrations are lower in the first and early second trimester than in normoglycemic nonpregnant women. Elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes which has already existed before the pregnancy (WHO. 1999). In 2013, the WHO divided hyperglycemia in pregnancy as follows: 1) diabetes in pregnancy, which means pregnancy occurring in a woman with known diabetes, overt diabetes first detected during pregnancy, or pre-gestational diabetes, and 2) GDM (WHO. 2014). Recently, the American Diabetes Association (ADA) suggested that women diagnosed with diabetes in the first trimester should be classified as having overt or preexisting pre-gestational diabetes, meaning T2DM or, very rarely, type 1 diabetes (T1DM). According to the ADA, GDM is diabetes that is first diagnosed in the second or third trimester of pregnancy and that is not clearly either preexisting T1DM or T2DM (American Diabetes Association. 2017).

The pathogenesis of GDM results mainly from two causes: increased insulin resistance (IR) and β-cell dysfunction (Buchanan & Xiang. 2005). IR is generally defined by a decrease in insulin sensitivity in the peripheral tissues (Hurrle & Hsu. 2017). Pregnancy is normally characterized by increased IR that begins near mid-pregnancy and progresses through the third trimester to levels that approximate the IR seen in individuals with T2DM (Catalano et al. 1999). Increased maternal IR is physiologically important, since carbohydrate is the major fuel for fetal growth (Catalano et al. 2003). IR during pregnancy seems to result from a combination of increased maternal adiposity and the insulin-desensitizing effects of hormonal products of the placenta. The fact that in the majority of GDM cases, glucose regulation will return to normal after delivery suggests that the major contributors to this state of resistance are placental hormones (Barbour et al. 2007). The second point is that pancreatic β-cells normally increase their insulin secretion to compensate for the IR of pregnancy (Buchanan & Xiang. 2005). However, various stressful stimuli, such as nutrient overload, advanced glycation, and oxidative stress followed by lipoxidation have been shown to lead to β-cell dysfunction (Sasson. 2017). Pregnant women with GDM tend to have greater IR than women with normoglycemic pregnancy (Catalano et al. 1991, Catalano et al. 1999). As a result, changes in circulating glucose levels over the course of pregnancy are relatively small compared with the large changes in insulin sensitivity. Strong β-cell function before increasing IR with advancing gestational age is the hallmark of standard glucose regulation during pregnancy (Buchanan & Xiang. 2005).
Other factors that may affect IR during pregnancy include body composition, the prevalence of metabolic syndrome (MetS), and other obesity-related chronic diseases (Cossrow & Falkner, 2004, Ervin, 2009). Further, there is evidence of a genetic association between common T2DM-risk gene variants with GDM (Mao et al. 2012). The published literature provides support for genetic variants having an effect on T2DM and β-cell function, but understanding of the genetic basis of IR remains more limited (Manning et al. 2012, Walford et al. 2016). One explanation for that could be that adiposity may hide the localization of genetic variants influencing IR by introducing extra variance in the outcome that is not attributable to genetic variation (Prudente et al. 2009). However, up to now few additional loci associated with fasting insulin and other IR-associated traits have been observed (Manning et al. 2012).

2.1.2 Diagnosis and prevalence

Insulin sensitivity increases in the first and early second trimester, and since both fasting and postprandial glucose levels are lower in early stages of pregnancy than in normoglycemic nonpregnant women, the diagnostic criteria of GDM are lower than those of DM (Diabetes. Current Care Guidelines. 2018, Gestational diabetes. Current Care Guidelines. 2013). While the earliest GDM criteria were based mostly on the future risk of developing diabetes, the more recent thresholds of GDM have been based on adverse perinatal outcomes (International Association of Diabetes and Pregnancy Study Groups Consensus Panel et al. 2010, Mishra et al. 2016).

In 1964, O'Sullivan and Mahan provided the first evidence that screening, diagnosis and treatment of glucose intolerance during pregnancy in women not previously known to have diabetes improved outcomes (O’Sullivan & Mahan. 1964). Based on data obtained from oral glucose tolerance tests (OGTTs) performed on 752 gravidas, the authors proposed the first diagnostic criteria for GDM based on the results of 3-hour (h) 100-gram (g) OGTTs, which were 5.0 mmol/L when fasting (f), and after a 100-g oral glucose intake 9.2 mmol/L at 1 h, 8.0 mmol/L at 2 h and 6.9 mmol/L at 3 h. O’Sullivan and Mahan published cutoff values based on whole-blood glucose values two standard deviations (SDs) above the mean at each of these time points, and an abnormal OGTT result was defined as two or more pathological values out of four (O’Sullivan & Mahan. 1964). Moreover, in 1973 O’Sullivan et al. first introduced a universal 50-g blood
glucose challenge test (GCT) with a cut-off value of 7.2 mmol/L in all pregnant
women. The sensitivity of the GCT was 79% and specificity 87% for GDM in a
population of 752 pregnant women, all of whom also underwent the diagnostic
100-g, 3-h OGTT (O’Sullivan et al. 1973). Nevertheless, the positive (PPV) and
negative predictive value (NPV) of the GCT depended greatly on the prevalence of
GDM in the studied population (Mishra et al. 2016).

In 1979 and 1982, the international panel of the National Diabetes Data Group
(NDDG), along with Carpenter and Coustan (C&C) recommended new diagnostic
cut-off values for the 100-g OGTT, both illustrated in Table 1 (Carpenter &
Coustan. 1982, NDDG. 1979). In addition, the WHO established uniform
definitions of diabetes for nonpregnant individuals in 1980, and extended this
recommendation to pregnant women (WHO. 1999). The NDDG first preferred
the use of plasma instead of whole blood for glucose analysis. Because the
concentration of plasma glucose is about 11–13% higher than in whole blood, the
glycemic cut-offs were raised by the NDDG (Holtkamp et al. 1975, NDDG. 1979).
The NDDG panel supported a two-step method, first with universal screening by
using the 50-g GCT, followed by a 100-g OGTT if the screen GCT was positive,
whereas the WHO proposed a one-step screening strategy by using two values, i.e.
fasting and 2-h plasma glucose levels in connection with the 2-h 75-g OGTT as

In 1998, the International Association of Diabetes and Pregnancy Study Groups
(IADPSG) was established to find universal agreement between many national and
international recommendations addressing diabetes in pregnancy. This
multinational delegation reviewed the data of the elaborate Hyperglycemia and
Adverse Pregnancy Outcome (HAPO) study (HAPO Study Cooperative Research
Group et al. 2008). In 2010, the IADPSG suggested universal screening with a
single-step approach and new diagnostic criteria for GDM that was based on a 2-h,
75-g OGTT. While all the earlier GDM criteria were based mostly on future risk of
developing diabetes, not on adverse perinatal outcomes (Mishra et al. 2016), the
new thresholds of the IADPSG were placed according to an 1.75 odds ratio (OR)
of having complications seen in the HAPO study (International Association of
Diabetes and Pregnancy Study Groups Consensus Panel et al. 2010). A basis on
adverse perinatal outcomes is the great advantage of IADPSG criteria, but one
criticism has been that it increases the number of GDM diagnoses, as a relatively
low cut-off value of fasting plasma glucose is used (Rani & Begum. 2016). Further,
at the beginning, a second limitation was that the HAPO study was performed
mainly among Caucasian women (Mishra et al. 2016). Later, it was proved that IADPSG criteria can be adopted for women of Indian origin (Seshiah et al. 2012).

Thus, after several decades of research there is still no global consensus on screening or diagnostic methods and criteria for GDM (Negrato & Gomes. 2013, Rani & Begum. 2016). In general practice, the WHO, for instance, has now adopted the IADPSG recommendations, whereas the American Congress of Obstetricians and Gynecologists (ACOG) advises continuing with the two-step screening procedure (The Committee on Obstetric Practice. 2011, WHO. 2013a). Currently, the ADA accept both the one- and two-step methods to screen and diagnose GDM, agreeing with the ACOG and IADPSG recommendations (Agarwal. 2015). Further, depending on the country, screening and diagnostic methods can be risk-based or universal one- or two-step procedures. The diagnosis of GDM is made by using 75-g or 100-g OGGTs. Risk factors of GDM include, for instance, obesity, previous GDM or a previous macrosomic infant weighing 4.5 kg or more, known history of DM in first-degree relatives, ethnic family origin (non-Caucasian women) with a high prevalence of DM, and clinical conditions associated with IR such as polycystic ovary syndrome (PCOS) (Gestational diabetes. Current Care Guidelines. 2013, Rani & Begum. 2016). However, there is evidence that 2.7–20 % of women diagnosed as having GDM have no risk factors for it (Avalos et al. 2013, Chevalier et al. 2011).

In Finland, GDM screening using a 75-g 2-h OGGT is offered to all pregnant women, except those who are at the lowest risk: primiparous women less than 25 years old and body mass index (BMI) 25 kg/m² or below and no known history of DM in first-degree relatives, or multiparous women less than 40 years old and no GDM in previous pregnancy or pregnancies and BMI 25 kg/m² or below before the current pregnancy (Gestational diabetes. Current Care Guidelines. 2013). Formal systematic testing is normally done between 24 and 28 weeks of gestation. However, the first screening is already offered at 12 to 16 gestational weeks for women at high risk of GDM. Factors indicating high GDM risk are GDM in previous pregnancy or pregnancies, BMI over 35 kg/m² before the pregnancy, glucosuria, T2DM in first-degree relatives, oral medication with glucocorticoids, and PCOS. To determine if GDM is present in pregnant women, a standard OGGTT is recommended after overnight fasting by giving 75 g anhydrous glucose in 250–300 ml water. Venous plasma glucose is measured in fasting samples, and after one and two hours (Gestational diabetes. Current Care Guidelines. 2013). The diagnostic criteria for GDM according to Finnish Current Guidelines and some of the most commonly used criteria worldwide are presented in Table 1 (Agarwal.
Table 1. Commonly used guidelines globally for the diagnosis of GDM.

<table>
<thead>
<tr>
<th>Organization</th>
<th>Year</th>
<th>Advice for screening</th>
<th>Method of screening (positive cut-off)</th>
<th>Glucose load, g</th>
<th>Glucose thresholds (mmol/L)</th>
<th>No. of OGTT values for diagnosis</th>
</tr>
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<tbody>
<tr>
<td>ACOG</td>
<td>2013</td>
<td>all except low risk</td>
<td>50 g GCT (≥ 7.8)</td>
<td>100</td>
<td>5.3</td>
<td>7.8</td>
</tr>
<tr>
<td>C&amp;C</td>
<td>1982</td>
<td>none</td>
<td>OGTT</td>
<td>100</td>
<td>5.3</td>
<td>7.8</td>
</tr>
<tr>
<td>CDA</td>
<td>2013</td>
<td>not specified</td>
<td>50 g GCT (≥ 7.8)</td>
<td>75</td>
<td>5.3</td>
<td>7.8</td>
</tr>
<tr>
<td>EASD</td>
<td>1991</td>
<td>not specified</td>
<td>OGTT</td>
<td>75</td>
<td>5.3</td>
<td>7.8</td>
</tr>
<tr>
<td>Finnish Guidelines</td>
<td>2013</td>
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<td>OGTT</td>
<td>75</td>
<td>5.1</td>
<td>7.8</td>
</tr>
<tr>
<td>NDDG</td>
<td>1979</td>
<td>none</td>
<td>50 g GCT (≥ 7.8)</td>
<td>100</td>
<td>5.8</td>
<td>8.0</td>
</tr>
<tr>
<td>NICE</td>
<td>2015</td>
<td>clinical risk</td>
<td>OGTT</td>
<td>75</td>
<td>5.6</td>
<td>7.8</td>
</tr>
<tr>
<td>WHO</td>
<td>1999</td>
<td>not specified</td>
<td>OGTT</td>
<td>75</td>
<td>7.0</td>
<td>7.8</td>
</tr>
<tr>
<td>WHO</td>
<td>2013</td>
<td>universal</td>
<td>OGTT</td>
<td>75</td>
<td>5.1</td>
<td>7.8</td>
</tr>
</tbody>
</table>


During the last decade, the prevalence of GDM has increased across the developed world, placing it as one of the most common metabolic complications of pregnancy (American Diabetes Association. 2014). Globally, the prevalence of GDM varies from 2% to 32%; a median estimate for North America is 9% and for Europe 6% (Zhu & Zhang. 2016). Recently, the prevalence of GDM has also quickly grown in Finland, being 17.5% in 2016 (Vuori & Gissler. 2017). The prevalence is increasing mostly because of the older age and higher BMI of gravidas. In Finland, the Current Guidelines were published in 2008 and updated in 2013 without any change in the diagnostic criteria of GDM. However, the Finnish diagnostic criteria and screening strategy of GDM were changed in 2008. At that time OGTT screening during pregnancy was extended from risk-based to consider...
all pregnant women, expect those at low risk (Gestational diabetes. Current Care Guidelines. 2013). The extended screening procedure might also have an affect on the increased prevalence of GDM in Finland. Figure 1 shows the prevalence of GDM in Finland in 2008–2016. Further, it presents both the mean age and BMI of parturients in Finland in the same time period.

The prevalence of GDM varies widely depending mostly on the population screened, different strategies for detection of GDM and the diagnostic test and criteria being used (Akgöl et al. 2017, American Diabetes Association. 2017, WHO. 2013a). For example, according to Akgöl et al. (2017), the new IADPSG criteria lead to a higher GDM prevalence and more diagnoses in young women when compared with other strategies (Akgöl et al. 2017).

Figure 1. Average age and BMI of parturients, and prevalence of GDM in Finland in 2008–2016 (Vuori & Gissler. 2017).

2.1.3 Long-term outcomes of mothers after gestational diabetes mellitus

Pregnancy has been said to be a window to the future health of a woman (Catov & Margerison-Zilko. 2016, Gilmore et al. 2015). Although in the majority of GDM
cases, glucose regulation will return to normal after delivery (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 1997), several studies have indicated that a diagnosis of GDM has significant implications for the future health of the mother. For instance, women with prior GDM have a higher risk of recurrence of GDM in future pregnancy, the rate of recurrence varying between 30 to 84% (Kim et al. 2002, Kim, Berger et al. 2007). GDM also appears to be associated with depressive symptoms shortly after delivery (Varela et al. 2017). Further, there is at least a sevenfold risk of T2DM after GDM (Bellamy et al. 2009).

In addition, studies reported earlier have shown a greater prevalence of metabolic syndrome in women with prior GDM (Y. Xu et al. 2014). Research data have also revealed subclinical inflammation and vascular dysfunction after GDM (Heitritter et al. 2005), contributing to a higher risk of cardiovascular disease (CVD) (Goueslard et al. 2016, Shah et al. 2008, Vrachnis et al. 2012). Postpartum glucose testing is important in screening for T2DM in women with previous GDM (Poola-Kella et al. 2017).

2.1.3.1 Type 2 diabetes mellitus

Although shortly after birth following GDM glucose tolerance is usually restored to pregestational levels, independent of population or ethnic group, affected women remain at an increased risk of developing type 2 diabetes mellitus (Ben-Haroush et al. 2004, Hunt & Schuller. 2007, Järvelä et al. 2006, Kim et al. 2002). The incidences of both GDM and T2DM are rising throughout the world, consequently resulting in huge health-care and economic costs (Hunt & Schuller. 2007, Lipscombe & Hux. 2007).

In 2002, Kim et al. published a review of 28 studies to examine the association between GDM and T2DM. They noticed that the cumulative incidence of T2DM after pregnancies complicated by GDM increased from 2.6% to over 70% when the follow-up of women was lengthened from 6 weeks to 28 years postpartum. The growth in incidence occurred markedly in the first five years after delivery and then plateaued after 10 years. During pregnancy, the level of fasting glucose was the factor which was most commonly associated with the risk of future T2DM (Kim et al. 2002). For instance, Steinhart et al. (1997) reported that the risk of future T2DM was increased 11-fold (OR 11.05; 95% CI 2.3–103.4), when the concentration of fasting glucose was over 5.83 mmol/L during pregnancy when compared with that in GDM women with lower levels (Steinhart et al. 1997).
Subsequently, Bellamy et al. published another, often-cited review in 2009. The meta-analysis of twenty studies, covering over 675,000 women with T2DM, confirmed undoubtedly the strong association between GDM and T2DM. According to Bellamy et al. (2009) women with earlier GDM have a relative risk (RR) of 7.43 (95% CI 4.79–11.51) of developing T2DM later in life when compared with women with previous normoglycemic pregnancies (Bellamy et al. 2009). Recently, research evidence revealed that among GDM women, both pregestational obesity and excessive weight gain from pre-pregnancy to the postpartum period magnifies the risk of T2DM after delivery (Liu et al. 2014). Further, decreased insulin sensitivity, β-cell compensation and recurrent GDM may contribute, and maternal factors such as lactation may reduce the risk of developing T2DM (Poola-Kella et al. 2017).

Unquestionably, the association between GDM and T2DM is strong. Further, the knowledge that several risk factors are the same suggests that these two disorders might have an overlapping cause (Kim et al. 2002). This hypothesis has been supported by the results of candidate gene studies (Y. M. Cho et al. 2009, Lauenborg et al. 2009).

For long periods of time, T2DM can be a silent disease leading to people being unaware of having the condition. Unfortunately, untreated disease is harmful due to the fact that both microvascular and macrovascular diabetic complications start to develop before typical symptoms of diabetes occur. The nature of T2DM is progressive, finally after many years of hyperglycemia culminating in end-organ damage and complications. Upon diagnosis of T2DM, about half of the pancreatic β-cell function is lost (Holman. 1998). In high-risk populations, including women with previous GDM, early detection of diabetes followed by necessary interventions may preserve β-cell function and reduce the risk of complications (DeFronzo & Abdul-Ghani. 2011). This is why women with prior GDM should be reclassified by means of OGTTs six weeks or more after delivery into one of the following categories: diabetes, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or normoglycemia (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 1997). In cases of medically treated GDM, medication is discontinued immediately after delivery. Finnish guidelines recommend OGTT screening six to twelve weeks after delivery in cases of medicated GDM during pregnancy, and one year after delivery in diet-treated GDM. If the first screen is abnormal (IFG or IGT), a subsequent OGTT test is suggested after one year (Gestational diabetes. Current Care Guidelines 2013). Moreover, if the screening result is normal, GDM women should undergo frequent
testing every three years by means of OGTTs for rest of their lives (Gestational diabetes. Current Care Guidelines. 2013, Kim, Herman et al. 2007, Metzger et al. 2007).

2.1.3.2 Metabolic syndrome

Metabolic syndrome (MetS) is an international health problem, the hallmarks of which are considered to be accumulation of abdominal obesity, hypertension, dyslipidemia and abnormal glucose tolerance or diabetes (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002). GDM shares common features with MetS, including dyslipidemia, insulin resistance and endothelial dysfunction (Anastasiou et al. 1998, Gobl et al. 2014, Hannemann et al. 2002, Heitritter et al. 2005, Isomaa et al. 2001). A variety of organizations have recommended slightly different definitions of MetS. These include the WHO, the National Cholesterol Education Program (NCEP) and the International Diabetes Federation (IDF) (Alberti & Zimmet. 1998, International Diabetes Federation. 2006, National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002). (There are more details in Section 2.2.1, below.)

In the 21st century, several investigators have explored the association between MetS and previous GDM (Table 2) (Akinci et al. 2011, Derbent et al. 2011, Di Cianni et al. 2007, Ijäs et al. 2013, Karoli et al. 2015, Lauenborg et al. 2005, Li et al. 2015, Mai et al. 2014, Noctor et al. 2015, Puhkala et al. 2013, Retnakaran et al. 2010, Tam, Ma, Yang et al. 2012, Verma et al. 2002, Wijeyaratne et al. 2006). Tam et al. (2007) reported similar rates of MetS in women with and without a history of GDM (7.5% vs. 8.1%; p = 0.85) followed up at a median of eight years (range 7–10) after delivery (Tam et al. 2007). Further, at a 5-year follow-up, Albareda et al. (2005) compared 262 women with former GDM with 66 normoglycemic controls. In accordance with NCEP ATP III criteria, women with a history of GDM differed only in the rate of fasting hyperglycemia and showed a trend toward a higher rate of hypertension, but the difference in prevalence of MetS (11.1% vs. 6.1%) was not significant (Albareda et al. 2005).
Table 2. Prevalence of MetS in women with and without prior GDM according to the current literature. Diagnostic criteria of MetS are shown in Table 3.

<table>
<thead>
<tr>
<th>Author and publication year</th>
<th>Number of GDM/ non-GDM</th>
<th>Treatment of GDM</th>
<th>Diagnostic criteria of GDM</th>
<th>Follow-up, years</th>
<th>Prevalence of MetS in GDM/non-GDM, %</th>
<th>Diagnostic criteria of MetS (see Table 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akinci et al. 2011</td>
<td>195/71</td>
<td>M</td>
<td>C&amp;C</td>
<td>3.4</td>
<td>25.1/5.6</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td>Derbent et al. 2011</td>
<td>36/40</td>
<td>NA</td>
<td>NDDG</td>
<td>4.1</td>
<td>52.8/7.5</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td>Di Cianni et al. 2007</td>
<td>166/98</td>
<td>M</td>
<td>C&amp;C</td>
<td>1.3</td>
<td>9.0/1.0</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td>Ijäs et al. 2013</td>
<td>61/55</td>
<td>M</td>
<td>Finnish guidelines</td>
<td>19</td>
<td>62.3/30.9</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td>Karoli et al. 2015</td>
<td>50/50</td>
<td>NA</td>
<td>ADA or C&amp;C</td>
<td>mean GDM 4.6/</td>
<td>64/10</td>
<td>IDF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nonGDM 4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lauenborg et al. 2005</td>
<td>457/987</td>
<td>D</td>
<td>Danish guidelines</td>
<td>9.8</td>
<td>43.5/14.8</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td>Li et al. 2015</td>
<td>1263/~</td>
<td>NA</td>
<td>WHO 1999</td>
<td>1–5</td>
<td>23.8/~</td>
<td>IDF</td>
</tr>
<tr>
<td>Mai et al. 2014</td>
<td>190/80</td>
<td>NA</td>
<td>ADA</td>
<td>mean GDM 2.5/</td>
<td>20/0</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nonGDM 2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noctor et al. 2015</td>
<td>265/378</td>
<td>NA</td>
<td>IADPSG</td>
<td>mean GDM 2.6/</td>
<td>25.3/6.6</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nonGDM 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puhkala et al. 2013</td>
<td>150/~</td>
<td>NA</td>
<td>Finnish guidelines</td>
<td>1</td>
<td>16 (18)/~</td>
<td>NCEP ATP III (IDF)</td>
</tr>
<tr>
<td>Retnakaran et al. 2010</td>
<td>137/259</td>
<td>NA</td>
<td>NDDG</td>
<td>3 months</td>
<td>19.7/10.0</td>
<td>IDF</td>
</tr>
<tr>
<td>Tam et al. 2012</td>
<td>45/94</td>
<td>NA</td>
<td>WHO 1999</td>
<td>15</td>
<td>22.2/14.9</td>
<td>IDF</td>
</tr>
<tr>
<td>Verma et al. 2002</td>
<td>58/51</td>
<td>NA</td>
<td>C&amp;C</td>
<td>11</td>
<td>27.2/8.2</td>
<td>NCEP ATP III</td>
</tr>
<tr>
<td>Wijeyaratne et al. 2006</td>
<td>147/67</td>
<td>NA</td>
<td>WHO 1999</td>
<td>3</td>
<td>49/6</td>
<td>IDF</td>
</tr>
</tbody>
</table>

**ADA**: American Diabetes Association; **C&C**: Carpenter & Coustan; **D**: diet only; **GDM**: gestational diabetes mellitus; **IADPSG**: International Association of the Diabetes and Pregnancy Study Groups; **IDF**: International Diabetes Federation; **M**: GDM cohort also includes medicated subjects; **MetS**: metabolic syndrome; **NA**: not available; **NCEP ATP III**: National Cholesterol Education Program Adult Treatment Panel III; **NDDG**: National Diabetes Data Group; **WHO**: World Health Organization

Recently, Xu et al. (2014) reported a meta-analysis (17 studies) demonstrating evidence of an increased risk of MetS after previous GDM. The odds ratio (OR) for MetS after GDM compared with normoglycemic pregnancy in BMI-matched groups was 2.53 (95% CI 1.88–3.41) (Y. Xu et al. 2014). Lauenborg et al. (2005) observed that obese women (BMI > 30 kg/m²) with previous GDM treated with diet only had a more than sevenfold higher prevalence of MetS when compared with normal-weight women after GDM (BMI < 25 kg/m²). Xu et al. (2014) also noticed that mothers with higher BMI had an elevated risk of MetS after GDM. Additionally, on average nineteen years after index pregnancies, Ijäs et al. (2013)
reported that pre-pregnancy overweight was the most powerful predictive component as regards developing MetS later in life. However, the risk of MetS was highest when both GDM and pre-pregnancy overweight were present (Ijäs et al. 2013). There is also evidence for an increased prevalence of MetS even among women who were normoglycemic when tested ten years after GDM, compared with controls (Lauenborg et al. 2005).

2.1.3.3 Cardiovascular disease

In women, atherosclerotic cardiovascular disease (CVD) remains the leading cause of death (S. K. Lee et al. 2017). While the association between GDM and T2DM is obvious, the link between GDM and CVD is more uncertain. Because of the time lag, typically two or three decades between GDM diagnosis and CVD events, epidemiological studies on the association are difficult to conduct. Further, such studies are greatly limited by the manner of ascertainment of GDM; universal screening and strategies for GDM are still missing (Kim. 2010a). However, the results of several studies suggest that GDM is an independent risk factor of CVD later in life (Fadl et al. 2014, Goueslard et al. 2016, Gunderson et al. 2014, Karoli et al. 2015, Lekva et al. 2017, Retnakaran & Shah. 2017), while other studies report that the raised prevalence of CVD risk is evident only in women who develop T2DM or abnormal glucose tolerance after GDM (Henry & Beischer. 1991, Kerenyi et al. 1999, Shah et al. 2008).

A review of four studies (n = 141 048) concerned the long-term risk of CVD when the time of follow-up ranged from 1.2 to 74.0 years. The risk of CVD among women with prior GDM varied between 0.28% and 15.5% (Hopmans et al. 2015). In a recent study on a population of 8127 North American women, CVD was diagnosed on average 22.9 years after a diagnosis of GDM. When multivariable-adjusted for socioeconomic, demographic, and lifestyle components including smoking habits, previous GDM was associated with 63% higher odds of CVD (OR 1.63; 95% CI 1.02–2.62; p = 0.04). However, the association became nonsignificant after additional adjustment for BMI (Shostrom et al. 2017). In a prospective cohort of 3416 women, GDM independently raised the risk of CVD (OR 1.26; 95% CI 0.95–1.68) (Fraser et al. 2012). Shah et al. (2008) found that women with previous GDM had a 70% increased incidence of CVD compared with women with earlier normoglycemic pregnancy, within just 11 years after the index pregnancy (Shah et al. 2008). Recently, within seven years postpartum, previous GDM was identified as an independent risk factor of CVD by Goueslard et al. They studied a database
of more than 1.5 million deliveries and found that the incidence of myocardial infarction was 0.04% in women with a previous diagnosis of GDM and 0.02% without (Goueslard et al. 2016). Further, Retnakaran and Shah (2017) reported a retrospective study of over 1.5 million women. Although the absolute rates of CVD events were very low, they noticed that women with a history of GDM had a higher risk of CVD events even in the absence of diabetes, but microvascular risk, including retinal and renal complications, emerged only in those women in whom T2DM developed (Retnakaran & Shah. 2017).

Mechanisms that contribute to a risk of CVD in women with previous GDM are mostly still uncertain. The fact that the risks of MetS and T2DM are increased after previous GDM naturally also contributes to the risk of CVD. Besides the chronic insulin resistance, β-cell failure and dyslipidemia, endothelial dysfunction is believed to be an important factor in the development of atherosclerosis after pregnancy complicated by GDM (Di Cianni et al. 2010, Landmesser et al. 2004). CVD risk postpartum seems to be potentiated by increased inflammatory markers among GDM women (Poola-Kella et al. 2017). There is also some evidence that adipokine imbalance in the presence of metabolic dysfunction may be a key event in promoting CVD (Lekva et al. 2017). Especially when combined with GDM, pre-pregnancy overweight has been shown to be an essential risk factor not only for subsequent diabetes, but also hypertension, which is a well-known traditional risk factor of CVD (Pirkola et al. 2010). In contrast, Gunderson et al. (2014) concluded that a history of GDM may be a marker of early atherosclerosis independent of pre-pregnancy obesity among women who have not developed T2DM or MetS (Gunderson et al. 2014).

Historically, medical trials on CVD prevention have been focused on men, and consequently there has been decreased awareness of the burden of CVD in women until recently. According to an interview survey, awareness of CVD risk increased among randomly selected women in the USA between 1997 and 2006 from 30% to 57%, but plateaued in 2009 (Mosca et al. 2010). Current literature shows that women with previous GDM have an increased risk of developing CVD later in life. At least in the absence of other recognized CVD risk factors, such as smoking, obesity and chronic hypertension, GDM is a useful marker of increased CVD risk (Fadl et al. 2014). It is very important that in daily practice GDM is recognized as a CVD risk factor unique to women.
2.1.4 Implications for clinical care

On a global basis, approximately 20 to 50% of people with T2DM remain undiagnosed. Early detection of T2DM is important, especially since treatment is proportionally economical and effective compared with treatment of later disease when management tends to be more complicated (International Diabetes Federation. 2011, Tong et al. 2008, Waugh et al. 2013). Knowing that women with GDM are at an increased risk of T2DM, the main focus of clinical practice should be on diminishing the risk of diabetes after pregnancy among these women. In addition, health care professionals should concentrate on detecting and treating diabetes that does develop. In the immediate postpartum period, determination of fasting glucose will identify women with impaired fasting glucose (IFG) in the diabetic range (Buchanan & Xiang. 2005). Moreover, all women should undergo OGTT screening at six weeks or later postpartum and, if screen-negative, have frequent testing for T2DM for rest of their lives (Gestational diabetes. Current Care Guidelines. 2013, Metzger et al. 2007). OGTT screening every three years seems to result in the lowest cost per case of detected diabetes (Kim et al. 2007)

Women with prior GDM are also at increased risk of recurrence of GDM in future pregnancy (Kim et al. 2002, Kim et al. 2007), so family planning is crucial to reduce the occurrence of unplanned pregnancies in the presence of glucose intolerance (Kjos et al. 1998). The increased proportion of preexisting diabetes, particularly among younger women early in their reproductive years, should also be of concern (Lawrence et al. 2008). Maternal hyperglycemia antedating pregnancy has implications for both maternal and infant health. If the presence of poor glucose control continues into the period of organogenesis, i.e. at 5–8 gestational weeks, women with preexisting diabetes expose their fetuses to a higher risk of congenital malformations and other complications (Lawrence et al. 2008).

Achieving a normal body weight is crucially essential to all GDM mothers after delivery (Gestational diabetes. Current Care Guidelines. 2013). Not surprisingly, the presence of both high maternal weight and GDM contribute to the risk of developing T2DM (Kaul et al. 2015). Consequently, women with both pregestational overweight or obesity and previous GDM require even more weight control after delivery. It has been suggested that pre-pregnancy weight and gestational weight gain are positively associated with women’s long-term cardiometabolic risks, including MetS, T2DM and CVD (Fraser et al. 2011, Liu et al. 2014, Willett et al. 1995). Hence, interventions that concentrate on reducing overweight and obesity should also be the focus of future public health care. This
would prevent or delay the onset of T2DM, and the risks of CVD or MetS in all women (Lawrence et al. 2008). Early postpartum lifestyle intervention should be taken to reduce the likelihood of postpartum weight gain and subsequent adverse cardiometabolic consequences (Li et al. 2015).

More effective public-health interventions aimed at prevention of T2DM are required, as well as enhanced resources to take care of the massive amount of individuals living longer with the disease (Lipscombe & Hux. 2007). Both epidemiological studies and clinical trials have revealed that the onset of T2DM in individuals at high risk can be delayed or even be prevented through lifestyle modifications such as diet and exercise, or pharmacological intervention including metformin, thus improving insulin sensitivity (Ben-Haroush et al. 2004, DeFronzo & Abdul-Ghani. 2011, Knowler et al. 2002, X. R. Pan et al. 1997, H. Tuomilehto et al. 2009, J. Tuomilehto et al. 2001). For example, after an average follow-up period of 2.8 years, metformin reduced the incidence of diabetes by 31% among subjects with impaired glucose tolerance (IGT) compared with placebo. In addition, the effect was even greater in those who were more obese, had higher fasting glucose or a history of GDM (Aroda et al. 2017). Further, metformin treatment for diabetes prevention has been estimated to be cost-saving (Aroda et al. 2017). In particular, targeting women with elevated levels of fasting glucose during pregnancy may have a considerable influence (Kim et al. 2002). Lifestyle interventions among the IGT population leading to at least a 5% reduction in weight have appeared to decrease the risk of T2DM by 58%, which is even more than treatment with metformin (Lindström et al. 2003). However, the changes in living may be hard to maintain.

GDM uncovers a β-cell defect persisting after pregnancy and typically becoming worse over time, increasing the risk of T2DM in the future. Further, coexisting obesity and incremental weight gain are additive elements as regards development to T2DM. Health care professionals including obstetricians play an important part in informing women with GDM about their lifelong risk of T2DM. In addition, primary health care should manage better in encouraging GDM women to participate in recommended screening and long-term follow-up after delivery (Durnwald. 2015). Although the importance of postpartum OGTT screening after GDM is known, rates of participation are alarmingly low, varying worldwide between 14 and 61 percent (Clark et al. 2009, Shea et al. 2011). Moreover, because GDM women, even before development of diabetes have significant differences in CVD risk factors, postpartum screening should not only be concentrated on glucose intolerance, but efforts should also be made to
minimize modifiable CVD risk factors, including hypertension, visceral adiposity, and dyslipidemia (Karoli et al. 2015).

2.2 Metabolic syndrome and obesity

Metabolic syndrome (MetS) is a term used to cover a cluster of metabolic and CVD risk factors including central adiposity, elevated BP, and abnormal lipid and glucose metabolism. Globally, MetS affects approximately one quarter of the adult population, women being influenced more often than men (Aguilar et al. 2015, Hess et al. 2017, International Diabetes Federation. 2006, Kaur. 2014, Mottillo et al. 2010, Shin et al. 2013). Among the MetS population, when compared with healthy controls, the chance of developing CVD is estimated to be six to eight times higher, and that of mortality related to CVD two to three times higher, the latter particularly among women (Gami et al. 2007, Haffner et al. 1998, Lakka et al. 2002, Sattar et al. 2003, Vanhala et al. 1997). Moreover, according to Hess et al. (2017), MetS is independently associated with a 70% increase in the risk of sudden cardiac death. Race or gender did not influence this association, which actually was even greater when the number of MetS components became larger. In particular, elevated BP, impaired fP-Gluc and low HDL-C drove this observed increased risk of sudden cardiac death (Hess et al. 2017). Furthermore, the longer the duration of MetS, the greater the risk of both DM and CVD (H. Hu et al. 2017, Ohnishi et al. 2016). Despite the above-mentioned research data, the clinical definition of MetS has sometimes been an issue of considerable debate (Balkau et al. 2002, Bauduceau et al. 2007, Borch-Johnsen & Wareham. 2010, Kahn et al. 2005, Mente et al. 2010, Simmons et al. 2010, Woodward & Tunstall-Pedoe. 2009).

Central obesity is one of the cardinal components of MetS. Generally, obesity means an excess of adipose tissue, and it can be assessed by body mass index (BMI) or waist circumference (WC) (Obesity (adult). Current Care Guidelines. 2013, Report of a WHO consultation. 2000). Obesity is related to endothelial dysfunction. Further, high BMI is correlated to a complicated interaction of inflammatory and metabolic features, and associated with a range of long-term disorders, disability, and decreased longevity (Berrington de Gonzalez et al. 2010, Fruhbeck et al. 2013, Global BMI Mortality et al. 2016, Meyers & Gokce. 2007). Research data have revealed that obesity raises the risk of both metabolic and cardiovascular (CV) diseases (Kopelman. 2000). In particular, visceral fat in comparison with subcutaneous fat is a more critical determining factor of CVD
risk and vascular structural modification (Lefferts et al. 2017). In clinical practice, measuring WC offers additional value to measuring BMI only (Tchernof & Despres. 2013).

### 2.2.1 Definitions and prevalence of metabolic syndrome

A number of organizations, including the WHO and the National Cholesterol Education Program (NCEP), have proposed somewhat different definitions of MetS. Regardless of which definition is used, the presence of MetS is believed to increase the risk of CVD at any concentration of LDL-C (Fruchart et al. 2004). MetS definitions for women according to WHO (Alberti & Zimmet. 1998), NCEP (Third report of the National Cholesterol Education Program 2001) and IDF (International Diabetes Federation. 2006) recommendations are presented in Table 3.

**Table 3. MetS definitions for women according to WHO, NCEP ATP III and IDF recommendations.**

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity</td>
<td>waist-to-hip ratio &gt; 0.85 and/or BMI &gt; 30 kg/m²</td>
<td>WC ≥ 88 cm</td>
<td>Increased (population-specific) WC</td>
</tr>
<tr>
<td>fP-Gluc, mmol/L</td>
<td>IGT, IFG or T2DM</td>
<td>≥ 6.1 or diabetes</td>
<td>≥ 5.6 or diabetes</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>≥ 140/90</td>
<td>≥ 130/85</td>
<td>≥ 130/85 or treatment for hypertension</td>
</tr>
<tr>
<td>TGs, mmol/L</td>
<td>≥ 1.7</td>
<td>≥ 1.7</td>
<td>≥ 1.7 or treatment for this lipid abnormality</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>≤ 1.0</td>
<td>&lt; 1.3</td>
<td>&lt; 1.3 or treatment for this lipid abnormality</td>
</tr>
<tr>
<td>Other</td>
<td>Microalbuminuria</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Definition</td>
<td>IGT, IFG, T2DM, or lowered insulin sensitivity + any 2 of the components</td>
<td>≥ any 3 of the components</td>
<td>Increased WC + any 2 of the components</td>
</tr>
</tbody>
</table>

BP: blood pressure; fP-Gluc: fasting plasma glucose; IDF: International Diabetes Federation; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; HDL-C: high-density lipoprotein cholesterol; MetS: metabolic syndrome; NCEP ATP III: National Cholesterol Education Program Adult Treatment Panel III; TGs: triglycerides; T2DM: type 2 diabetes mellitus; WC: waist circumference; WHO: World Health Organization

In recent decades, the prevalence of MetS has rapidly increased in parallel with sedentary lifestyles (Y. Xu et al. 2014). Nowadays, MetS is a major health problem affecting about 25% of the adult population worldwide (Kaur. 2014). Although
globally MetS affects women more often than men, MetS was present in about 22% of the women and 39% of the men in the middle-aged FINRISK cohort in 2004 (Ilanne-Parikka et al. 2004). The prevalence of MetS is almost double according to the IDF classification vs. that of the NCEP ATP III, because of the stricter values of fP-Gluc and abdominal obesity in the former. However, the NCEP ATP III classification better identifies the presence of insulin resistance (IR) than that of the IDF (Castro Dufourny et al. 2009).

2.2.2 Classification and prevalence of obesity

Body mass index is commonly used to classify both under- and overweight conditions and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m$^2$) (Report of a WHO consultation. 2000). Classification of overweight and obesity according to BMI is set out in Table 4 (Report of a WHO consultation. 2000). The classification is in agreement with that suggested by the WHO earlier (Report of a WHO Expert Committee. 1995), and is based primarily on the association between BMI and mortality (Report of a WHO consultation. 2000). Briefly, individuals having a BMI of at least 25 and under 30 kg/m$^2$ are overweight, and those having a BMI over 30 kg/m$^2$ are obese. Further, waist circumference (WC) is practical in clinical use when estimating central obesity. WC over 100 centimeters in men and over 90 cm in women increases the risk of death and comorbidity (Obesity (adult). Current Care Guidelines. 2013).

Table 4. Classification of overweight and obesity in adults according to BMI.

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m$^2$)</th>
<th>Risk of comorbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18.5 – 24.9</td>
<td>Average</td>
</tr>
<tr>
<td>Overweight</td>
<td>≥ 25.0</td>
<td></td>
</tr>
<tr>
<td>pre-obese</td>
<td>25.0 – 29.9</td>
<td>Increased</td>
</tr>
<tr>
<td>obese grade I</td>
<td>30.0 – 34.9</td>
<td>Moderate</td>
</tr>
<tr>
<td>obese grade II</td>
<td>35.0 – 39.9</td>
<td>Severe</td>
</tr>
<tr>
<td>obese grade III</td>
<td>≥ 40.0</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

According to Non-Communicable Diseases (NCD) Risk Factor Collaboration (2016), the global prevalence of obesity varies from 11% to 15% (NCD Risk Factor Collaboration (NCD-RisC). 2016). In contrast, according to the WHO, obesity is observed in 30% of the world population (WHO. 2015). In developed
countries, the prevalence of overweight and obesity is high and still increasing; the proportion of overweight women increased from 30% in 1980 to 38% in 2013 (Flegal et al. 2012, Ng et al. 2014). In the USA in 2009–2010, among women between 20 to 39 years of age 55.8% (95% CI 49.6–61.9) were overweight or obese (Flegal et al. 2012). According to the FINRISK 2012 study, over a half of the adult population are overweight and every fifth adult is obese in Finland. The prevalence of obesity among Finnish women was 20%, and that of an overweight condition 46% in 2012 (Borodulin et al. 2014).

2.2.3 Challenges of obesity in health care

Aging is associated with a decrease in resting metabolic rate; a decline in basal metabolism with age can be 1–2% per decade (Keys et al. 1973, Valiani et al. 2017). Therefore, weight usually increases with age, culminating in middle age (Peltonen et al. 2008). The prevalence of obesity has risen among Finnish men since the 1970s and among women since the 1980s. The fundamental cause of obesity is an energy imbalance between calories consumed and expended. Globally, there has been an increased intake of energy-dense food and a decrease in physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation and increasing urbanization (Hruby & Hu. 2015, WHO. 2013b). The growing prevalence of obesity seems to have plateaued during the last decade. In Finland, there is less obesity among adults living near the region of the capital, and in those areas with higher education (Borodulin et al. 2014, Peltonen et al. 2008). An overload of adipose tissue increases an individual’s risk of several comorbidities such as T2DM, CV and metabolic diseases, osteoarthritis, gout, asthma, sleep apnea, liver and renal diseases, depression, dementia and several types of cancer (Guh et al. 2009, Kivimäki et al. 2017, Obesity (adult). Current Care Guidelines. 2013).

Treatment of obesity should be provided in primary health care. The aim is to prevent comorbidities of obesity through at least a stable 5% reduction in weight, which seems to decrease the risk of T2DM by 58% (Lindström et al. 2003). The main element in therapy is lifestyle counseling on diet and physical activity. Lifestyle changes are supported with very-low-energy diets and medication, such as orlistat. If appropriate conservative treatments do not lead to sufficient weight loss, bariatric surgery is indicated in cases of morbid obesity (Obesity (adult). Current Care Guidelines. 2013). In Finland, obesity and its comorbidities represent a huge

Because of the health risks and significant increase in prevalence worldwide in recent decades, obesity has also become a major global health challenge. In contrast to other major global risks, there is little evidence of successful population-level intervention strategies to reduce the increasing incidence (Ng et al. 2014). For example, there is an ongoing global prevention program, incorporated into the WHO’s strategy, targeted at a 25% decrease in mortality caused by obesity by 2025. Despite this worldwide 25 × 25 strategy, the population mean for BMI has continued to increase, with a minor sign of plateauing (Kivimäki et al. 2017, NCD Risk Factor Collaboration (NCD-RisC). 2016, Pearce et al. 2014, WHO. 2013b). In contrast, long-term follow-up in the randomized Finnish Diabetes Prevention Study (DPS) revealed that lifestyle intervention among individuals at a high risk of T2DM induces a more permanent lifestyle change, resulting in long-term prevention of progression to T2DM (Lindström et al. 2013). In addition, bariatric surgery using the gastric bypass technique is an effective treatment for severe obesity, with long-term durability of weight loss, remission and prevention of comorbidities, and an improved quality of life (Adams et al. 2017, Nguyen et al. 2017). Further, a randomized controlled trial exposed the positive effect of a weight-management program delivered by social media on weight and risk features of MetS among overweight and obese adults. The participants in the Facebook Group reported a 4.8% reduction in initial weight after 24 weeks of follow-up when compared with a control group (Jane et al. 2017). However, like many other trials on obesity management, this study is too short to allow conclusions on possible long-term benefit.

In conclusion, obesity impairs the health of people and results in enormous financial costs, and, furthermore, it decreases working ability and the quality of life among the affected population. Since over 50% of the Finnish adult population are at least overweight (Borodulin et al. 2014), the prevention of obesity is a great challenge in public health. Increasing rates of overweight and obesity both in childhood and among adolescents should also be of concern, because overweight and obesity in childhood is usually maintained in adulthood (A. S. Singh et al. 2008).
2.3 The atherosclerotic process

Atherosclerosis is a chronic process that begins at an early age and is progressive in nature, leading to the development of both CV and cerebrovascular diseases (Furie & Mitchell. 2012, C. J. Lee & Park. 2014, Rocha & Libby. 2009). Atherosclerotic lesions are not associated with any symptoms at an early stage, but their initial presentation may result in catastrophic CVD events such as myocardial infarction and stroke resulting from plaque rupture and thrombosis (Giroud et al. 1992, Schroeder & Falk. 1995). The clinical manifestations of atherosclerotic disease depend on the site of the plaque (Dwivedi et al. 2018, R. B. Singh et al. 2002). Atherosclerotic plaque formation is illustrated in Figure 2 and it involves: 1) low-density lipoprotein (LDL) accumulation in the intima; 2) oxidation of LDL; 3) recruitment of circulating monocyte-derived macrophages; 4) uptake of oxidized LDL (oxLDL) by macrophage scavenger receptors, and transformation of macrophages into foam cells; and 5) formation of a fibrous cap containing smooth muscle cells, which permits stabilization of the plaque (Tedgui & Mallat. 1999). Buildup of plaques narrows the lumen of arteries, restricting blood flow to organs and tissues, leading to ischemia (Schroeder & Falk. 1995).

**Figure 2.** Process of atherosclerotic plaque formation.

LDL: low-density lipoprotein; MMP: matrix metalloproteinase; oxLDL: oxidized low-density lipoprotein; ROS: reactive oxygen species; SMC: smooth muscle cell.
Nowadays, there is evidence that chronic inflammation and increased oxidative stress are important elements of atherosclerosis (Feng et al. 2011, Kattoor et al. 2017, Stocker & Keaney. 2004). Oxidative stress means imbalance in favor of increased generation of reactive oxygen species (ROS) and/or reduced native antioxidant defense systems of the body (Peluso et al. 2012). Reactive oxygen species play an essential role in inflammatory responses, cell growth, and apoptosis. Locally, the role of ROS is crucial when altering vascular tone as well as initiating oxidation of LDL (Figure 2). Oxidized LDL is considered more important in atherogenesis than innate LDL (Zhang & Gutterman. 2007). In addition, the process of intimal calcification has long been associated with coronary atherosclerosis (Dwivedi et al. 2018).

2.3.1 Low-density lipoprotein particles in the arterial wall

In humans, the first visible lesion of atherosclerosis is called the foam cell. These foam cells are primarily derived from arterial-wall macrophages with accumulated lipoproteins, particularly low-density lipoproteins (LDLs) (Steinberg. 2009). Circulating monocyte-derived macrophages cannot take up native LDL rapidly enough to cause lipid loading (Goldstein et al. 1979). However, a high plasma concentration of LDL increases the transportation of LDL particles in the intima of arterial walls. In the intima of arteries, in other words in the subendothelial space, LDL may undergo oxidative modification. Oxidized LDL is considered to be atherogenic, and this oxidation process represents one of the first steps of the atherosclerotic process (Bowie et al. 1993, Steinberg. 1988, Stocker & Keaney. 2004). Smooth muscle cells and endothelial cells in lesions can also load lipid droplets, but foamy macrophage formation predominates (Steinberg. 2009). Besides oxidative modification, LDL particles may also undergo glycosylation, which consequently increases their susceptibility to oxidation. Thus, glycosylation of LDL partly explains the increased incidence of atherosclerosis in individuals with DM (Bowie et al. 1993).

Reverse cholesterol transport is a pathway defined as the transportation of accumulated cholesterol from the vessel wall to the liver for excretion, thus preventing atherosclerosis. Major components of reverse cholesterol transport include acceptors such as high-density lipoprotein (HDL) and apolipoprotein A-I, and enzymes such as lecithin cholesterol acyl transferase (Ohashi et al. 2005, Small. 1988). The protective effects of HDL are mediated by cell-surface HDL receptors,
and HDL may function as an acceptor, transporter and inactivator of oxLDLs (R. B. Singh et al. 2002).

2.3.2 Risk factors of atherosclerosis

Atherosclerosis is a multifactorial disease involving the interplay of genetic and environmental factors (R. B. Singh et al. 2002). In accordance with the fact that oxidative stress and inflammation are important features in the development of atherosclerosis, the risk factors are commonly associated with excess production of reactive oxygen species and oxidation of LDL in the vessel wall (Förstermann et al. 2017). In the general population, the impact of traditional risk factors such as age, sex, family history, obesity, hypertension, smoking, high levels of LDL cholesterol (LDL-C), and low levels of HDL cholesterol (HDL-C) on CVD has long been established beyond any doubt (Faxon et al. 2004, Fruchart et al. 2004). Further, several studies have shown that raised levels of triglycerides (TGs) are associated with increased CVD risk (Yarnell et al. 2001). Additionally, many novel risk factors of the atherosclerotic process have been recognized in recent decades. It is important to identify individuals at a raised risk of CVD, and consequently, modify their risk factors early on. Also, the treatment of advanced atherosclerosis is less effective than inhibition of atherosclerosis progression (Insull. 2009).

2.3.2.1 Traditional risk factors

Conventional CVD risk factors include age, male gender, high concentrations of LDL-C, elevated blood pressure, smoking, and further, family history, obesity, physical inactivity and a high-fat diet (Bertoluci & Rocha. 2017, Faxon et al. 2004, Fruchart et al. 2004, Martin-Timon et al. 2014, Vogel. 1997). Age is the most powerful non-modifiable risk element of CVD. Gender aside, growth in CVD risk with the level of each risk factor is continuous and progressive (Bertoluci & Rocha. 2017). In general, the age-adjusted incidence of a new myocardial infarction is higher in men than in women, with a hazard ratio (HR) of 2.56 (95% CI 2.53–2.60) (Booth et al. 2006). In individuals with DM, the difference between genders is narrower, but still higher in men. However, women with DM seem to have a greater relative risk than diabetic men when considering the rate of mortality from coronary causes (Haffner et al. 1998, Huxley et al. 2006). In a meta-analysis of 37 prospective cohort studies, the rate of fatal coronary heart disease was substantially
higher in people with diabetes than in those without (5.4% vs. 1.6%). This difference was even more apparent among women with and without DM (7.7% vs. 1.2%) than among men with and without DM (4.5% vs. 2.0%) (Huxley et al. 2006). In addition, a family history of CVD, generalized obesity determined by BMI and abdominal obesity assessed by waist circumference (WC) as well as a high-fat diet are associated with a higher risk of CVD (Martin-Timon et al. 2014, Pandey et al. 2013, Vogel. 1997, Weir. 2007). On the other hand, regular physical exercise has long been correlated with a lower risk of CVD morbidity and mortality, and there may simultaneously be other positive aspects of a lifestyle including regular physical activity (Powell et al. 1987, Shephard & Balady. 1999).

Hypercholesterolemia means elevated levels of cholesterol in the blood, which can be a result of either monogenic (such as familial hypercholesterolemia) or polygenic inheritance, or environmental factors (Taylor et al. 2017). Hypercholesterolemia is a strong and independent risk factor of CVD mortality, which is potentiated by diabetes. Further, LDL-C is one of the most important reversible risk components of CVD morbidity and mortality (Stamler et al. 1993). When reducing levels of LDL-C by 1 mmol/L via statin therapy, the RR of CVD will decrease by 20% (Cholesterol Treatment Trialists’ (CTT) Collaborators et al. 2008). This phenomenon is linear and it is likely to occur similarly at any level of baseline LDL-C, at least down to 1.3 mmol/L. In individuals with DM, per each mmol/L of reduction in concentrations of LDL-C, statin therapy brings about a relative reduction of 9% in total mortality (p = 0.02) and a 21% reduction in the incidence of major CVD events (p < 0.0001) such as acute myocardial infarction (AMI) and stroke. In addition, there are also significant changes in coronary revascularization (Cholesterol Treatment Trialists’ (CTT) Collaborators et al. 2008).

Cigarette smoking is one of the most important reversible risk factors of CVD. Compared with women who have never smoked, the incidence of AMI is raised sixfold in women who smoke at least 20 cigarettes per day (Njolstad et al. 1996). In a meta-analysis of 46 studies, including approximately 130 000 patients with DM, the RR of smokers compared with nonsmokers was 1.48 (95% CI 1.34–1.64) for total mortality, 1.36 (95% CI 1.22–1.52) for CVD mortality, 1.54 (95% CI 1.31–1.82) for CVD events, 1.44 (95% CI 1.28–1.61) for stroke and 1.52 (95% CI 1.25–1.83) for AMI (Qin et al. 2013). Among diabetic individuals, active smoking is correlated with the greatest risk of total mortality and CVD events, whereas finishing smoking is associated with a decreased risk in both. A large meta-analysis of 89 cohorts was carried out to evaluate the effect of active smoking on mortality. Comparing participants who were active smokers with former smokers and those
who had never smoked, active smoking was associated with more than 50% growth in mortality and CVD events in comparison with nonsmokers. However, former smokers were at a higher risk of mortality and CVD events than individuals who had never smoked. Among patients with DM, there is a crucial advantage in stopping smoking, but a major remnant risk, which seems to be proportional to the exposed time of smoking, indicating that smoking should be stopped as early as possible (A. Pan et al. 2015).

Hypertension, i.e. elevated blood pressure (BP), affects all parts of the CV system and is a well-verified risk element of CVD (Koller. 2002). At all ages, isolated systolic hypertension is an important CVD risk factor, both in women and men (James et al. 2014). In the Framingham study, diastolic BP was the most powerful predictor of CVD risk in individuals of less than 50 years of age. In patients aged between 50 and 59 years, all parameters of BP were prognostic for CVD, whereas in those more than 60 years old, pulse pressure (PP) had the strongest prognostic value (Lloyd-Jones et al. 1999). In both T1DM and T2DM, hypertension is a remarkable risk component as regards microvascular complications and atherosclerotic CVD events. In T1DM, hypertension is commonly the result of underlying diabetic kidney disease, while in T2DM, it usually coexists with other cardiometabolic risk elements (American Diabetes Association. 2016). In a recent review of 40 studies, including over one hundred thousand adults with T2DM, lowering of systolic BP was evaluated. Research data revealed that for each 10 mmHg drop in systolic BP there were significant decreases in the risks of many outcomes such as: mortality (RR: 0.87; 95% CI 0.78–0.96), CVD events (RR: 0.89; 95% CI 0.83–0.95), coronary heart disease (RR: 0.88; 95% CI 0.80–0.98) and stroke (RR: 0.73; 95% CI 0.64–0.83) (Emdin et al. 2015). In 2016, the American Diabetes Association (ADA) recommended a goal of 140 mmHg for systolic BP and 90 mmHg for diastolic BP when treating people with DM and hypertension (American Diabetes Association. 2016). In Finland, BP under 140/80 mmHg is a target for diabetic individuals (Diabetes. Current Care Guidelines. 2018).

Evidently, classic CVD risk factors such as a high serum cholesterol level, cigarette smoking, and elevated BP are significant predictors of CVD mortality. Further, these three major risk factors have been shown to have an additive influence on CVD mortality. In a cohort of over 347 000 men, age-adjusted CVD death rates progressively increased with an increasing number of these three major risk factors. The relative risk of CVD death was 2.0 for non-diabetic men with any one factor only, 3.7 for those with any two only, and 7.9 for those with all three
Moreover, the presence of risk factors, separately or in combination, was associated with an even more progressive increase in CVD mortality among diabetic vs. non-diabetic men (Stamler et al. 1993).

2.3.2.2 Insulin resistance

Insulin maintains euglycemia via transporting glucose from the circulation into the muscles and other tissues (Dongerkery et al. 2017). Additionally, insulin pushes glucose conversion into glycogen in the liver and skeletal muscle, promotes accumulation of TGs in adipose tissue, and downregulates significant gluconeogenic enzymes in the liver (Choi et al. 2010). Dysregulation of insulin signaling may result in IR, where the ability of cells to respond to the action of insulin is diminished, leading the pancreas to synthesize more insulin. As long as anyone can produce enough insulin to overcome IR, plasma glucose levels remain normal. Once the pancreas is no longer able to keep up, levels of plasma glucose begin to rise. IR is the earliest feature in the pathogenesis of T2DM and it develops in multiple organs including skeletal muscle, liver, adipose tissue and the heart (Stafeev et al. 2017). Hyperinsulinemia, as a result of IR, occurs before diagnosis of T2DM (Mitsuhashi et al. 2011, Muntoni et al. 2008, Pyörälä. 1979, Stout. 1990). Further, IR and arterial stiffness are interrelated, leading to increased CVD morbidity and mortality (Westerbacka & Yki-Järvinen. 2002). The onset of hyperglycemia and DM is generally antedated by many years of IR. Insulin favors abdominal obesity, which actually plays an important part in IR (Bhatia et al. 2012, Dongerkery et al. 2017). Further, this phenomenon provides an important link between T2DM and the accumulation of fat (Bhatia et al. 2012). Consequently, a negative vicious circle is completed when a major proportion of individuals with T2DM are obese (Hossain et al. 2007).

There are several methods to measure IR. At present, the hyperinsulinemic euglycemic clamp remains a gold standard for accurately determining IR, but due to the invasive and time-consuming technique, it is not implemented on a routine basis (DeFronzo et al. 1979, Gutch et al. 2015, Park et al. 2015). Therefore, some more simple methods have been validated for clinical practice. For example, the quantitative insulin sensitivity check index (QUICKI) and homeostasis model assessment of insulin resistance (HOMA-IR) are suitable for clinical use (Gutch et al. 2015). The latter, HOMA-IR, was first developed in 1985 by Matthews et al., and for now, it has proved to be a robust clinical and epidemiological tool for the assessment of IR (Antuna-Puente et al. 2011, Lann & LeRoith. 2007, D. R.
Matthews et al. (1985). HOMA-IR involves use of fasting plasma glucose (fP-Gluc) and insulin (fP-Insu) levels to quantify both IR and β-cell function. A final result is mathematically derived from use of the insulin-glucose product: fP-Gluc × fP-Insu, divided by 22.5 (D. R. Matthews et al. 1985).

2.3.2.3 Dyslipidemias

Lipoproteins are macromolecular complexes consisting of core lipids, which mainly are TG and cholesteryl esters, surface phospholipids, free cholesterol, and one or more apolipoproteins. Based on physical characteristics, molecular weight, diameter, and chemical composition, lipoproteins can be divided into five classes including chylomicrons, very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), which may also be referred to as remnants of VLDL, LDL, and HDL (Ginsberg, 1998, Gotto et al. 1986). Dyslipidemias include disorders of lipoprotein metabolism leading to the overproduction of potentially atherogenic lipoproteins, LDL, VLDL and IDL. Furthermore, there may be a decrease in the levels of HDL and an increase in the levels of small dense LDL particles (Chang & Robidoux. 2017). Dyslipidemias, in particular hypercholesterolemia, are common clinical conditions (Sanin et al. 2017). In the general population, high levels of total cholesterol (TC), LDL-C, and TGs, and low levels of HDL-C represent all essential determinants of atherosclerotic CVD (Mikolasevic et al. 2017). In the artery wall, HDL acts as a protector against LDL oxidation, and therefore high levels of HDL-C have an inverse relationship as regards the risk of atherosclerotic clinical events (Berliner et al. 1995). Further, non-HDL-C is a strong and independent predictor of CVD. It is more strongly associated with subclinical atherosclerosis than all other conventional lipid values. Non-HDL-C is defined as TC minus HDL-C (Orakzai et al. 2009).

Treatments to normalize dyslipidemias and reduce the risk of CVD events include both lifestyle modifications and medication (Khavandi et al. 2017). Reducing LDL-C has been the main therapeutic target to diminish the risk of CVD. Cholesterol-lowering types of medication, particularly statins, have been used to provide both primary and secondary prevention of CV conditions for many years (Sanin et al. 2017). Lately, lipid management has continued to evolve. Beyond maximum statin therapy among high-risk populations, ezetimibe further reduced LDL-C levels in cases of CVD (Khavandi et al. 2017). Further, LDL-C reduction may also be achieved by inhibition of the enzyme proprotein convertase subtilisin/kexin type-9 (PCSK9). Other treatments, more focused on TGs, are less
well supported by the results of randomized clinical trials and should be used on an individual basis. Up to now, trials aimed at pharmacologically increasing plasma HDL concentrations have failed to prevent CVD events. Some still-ongoing trials are focused more on HDL functionality and not just the absolute levels of HDL-C (Kampangkaew et al. 2017, Khavandi et al. 2017).

2.3.2.4 Other non-traditional biomarkers of increased risk: oxidized low-density lipoprotein, high sensitivity C-reactive protein and matrix metalloproteinase-8

Atherosclerosis begins with accumulation of lipoproteins, particularly low-density lipoprotein, in the arterial wall, where they are then subjected to oxidative modifications (Stocker & Keaney. 2004). Oxidized low-density lipoprotein (oxLDL) is a possible inflammatory molecule inducer and is considered to be the typical atherogenic form of LDL (Catapano et al. 2000, Steinberg. 2009). Circulating oxLDL seems to reflect the level of local atherosclerotic oxidative stress (Sigurdardottir et al. 2002). Further, increased amounts of circulating oxLDL are associated with the occurrence of coronary heart disease (Holvoet et al. 1998, Holvoet et al. 2001). There is also accumulating evidence that T2DM is associated with increased oxidative stress (Njajou et al. 2009, Odegaar et al. 2016). Oxidized LDL, when accumulating in the arterial wall, injures its endothelium, leading to endothelial dysfunction (Stocker & Keaney. 2004). Endothelial dysfunction leads to impaired arterial elasticity at an early stage in the atherosclerotic process (Cohn. 1999). Thus, both in the prevention of and therapeutic intervention in the atherosclerotic process, lowering concentrations of LDL-C and, consequently, inhibiting LDL oxidation have become an important focus (Parthasarathy et al. 1992, Ridker et al. 2009).

Concurrently with accumulation of oxLDL, inflammation may develop, which is a significant predictor of CVD complications (Faxon et al. 2004, Ridker et al. 2002). High-sensitivity C-reactive protein (hsCRP) is a known acute-phase protein and a sensitive biomarker of chronic low-grade systemic inflammation. An elevated concentration of hsCRP has been shown to be a strong risk factor as regards atherosclerosis, with an additive value in predicting CVD risk with extra atherothrombotic complications on top of traditional risk factors (Karadeniz et al. 2015, Ridker et al. 2002, van der Meer et al. 2002, Yamashita et al. 2003). Further, recent drug trials focusing on reduction of hsCRP have shown that decreasing the levels of hsCRP with rosuvastatin or canakinumab significantly reduced the incidence of major CVD events (Ridker et al. 2008, Ridker et al. 2017). The
pathogenicity of low-grade inflammation may also be mediated by inducing vascular dysfunction (Heitritter et al. 2005, Meigs et al. 2004).

**Table 5.** The location of production and functions of hsCRP, MMP-8, MMP-9 and TIMP-1 according to the literature (Brew & Nagase. 2010, Craig et al. 2015, Kamath et al. 2015, Kormi et al. 2017, Y. S. Lee et al. 2009, Pepys & Hirschfield. 2003).

<table>
<thead>
<tr>
<th>Variable of low-grade inflammation</th>
<th>Synthesized mainly by</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP</td>
<td>hepatocytes</td>
<td>Acute-phase reactant: elevated in response to acute infections, inflammatory conditions and trauma Predictive value in T2DM, MetS, increased carotid intima-media thickness, CVD</td>
</tr>
<tr>
<td>MMP-8</td>
<td>polymorphonuclear cells; at lower levels by lymphocytes, chondrocytes, lung epithelial, dendritic, mesenchymal stem, endothelial, smooth muscle and natural killer cells, fibroblasts, fibrocytes, activated monocytes and macrophages</td>
<td>Involved in wound healing and tissue remodeling during inflammation Capable of digesting extracellular matrix components Implicated in the pathogenesis of several chronic inflammatory diseases including cystic fibrosis, rheumatoid arthritis, periodontal disease, and chronic skin wounds Present within atherosclerotic lesions</td>
</tr>
<tr>
<td>MMP-9</td>
<td>leukocytes, fibroblasts, macrophages, epithelial and endothelial cells</td>
<td>Degrades extracellular matrix proteins including gelatin, collagen, elastin, and laminin Modulates the activities of other proteases, growth factors, cytokines and chemokines through proteolytic cleavage Tissue destruction and remodeling, inflammation</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>cardiac myocytes, fibroblasts, endothelial and smooth-muscle cells, monocytes and macrophages</td>
<td>The most important endogenous inhibitor of MMPs Various biological activities including modulation of cell proliferation, cell migration and invasion, anti-angiogenesis, anti- and pro-apoptosis and synaptic plasticity Potential role in inflammatory response</td>
</tr>
</tbody>
</table>

CVD: cardiovascular disease; hsCRP: high sensitivity C-reactive protein; MetS: metabolic syndrome; MMP: matrix metalloproteinase; T2DM: type 2 diabetes mellitus; TIMP: tissue inhibitor of metalloproteinase

The group of matrix metalloproteinases (MMPs) contains over 20 structurally and functionally involved but genetically distinct members (Lenglet et al. 2013, Sorsa et al. 2006). Normally, both expression and activity are low, but they are increased in several pathophysiological circumstances. MMPs can modulate immunological responses, and can be either defensive or destructive (Sorsa et al. 2006). Both upregulation and downregulation of MMP-8 and -9 have been
associated with many noninfectious as well as infectious inflammatory conditions (Lauhio, Salo et al. 1994, Lauhio, Konttinen et al. 1994, Lauhio et al. 1995, Lauhio, Saikku et al. 2011, Lauhio, Hastbäcka et al. 2011, Lauhio et al. 2016, Rautelin et al. 2009). MMPs have also been implicated in the formation of atherosclerosis and its progression in humans (Goncalves et al. 2009, Paim et al. 2013, Siasos et al. 2012). The major regulators of MMP activity are tissue inhibitors of matrix metalloproteinases (TIMPs), TIMP-1 being the most potent and well-studied of the four major endogenous inhibitors (Brew & Nagase. 2010). Circulating TIMP-1 has also been reported to be an independent predictor of CVD events and cardiac death (Cavusoglu et al. 2006, Lubos et al. 2006). The imbalance between MMP-8 and TIMP-1 may play a part in vulnerability of the atherosclerotic plaque to rupture, indicating an important role in CVD risk (Goncalves et al. 2009, Pussinen et al. 2013, Sorsa et al. 2011, Tuomainen et al. 2007). In summary, Table 5 illustrates the location of production and functions of hsCRP, MMP-8, MMP-9 and TIMP-1.

2.4 Arterial dysfunction

The endothelium – once considered only a semipermeable barrier separating the lumen from the vessel wall – has already long been recognized as an essential endocrine organ responsible for a variety of physiological processes crucial for vascular homeostasis (Vane et al. 1990, Vanhoutte. 1989). Endothelial cells not only transduce several physiological stimuli, but also produce numerous signaling molecules that exert both paracrine and autocrine effects. These include the regulation of vascular tone, luminal diameter and blood flow, hemostasis and thrombosis, inflammatory processes, vessel-wall interactions with both platelets and leukocytes, and control of vascular permeability, tissue growth and remodeling (Lane et al. 2006). The balance between vasoconstriction and vasodilatation is mostly controlled by the interaction between the vascular smooth muscle layer and endothelium-derived vasoactive mediators. As such, endothelial nitric oxide (NO) is a powerful vasodilator and one of the most significant controllers of vascular tone (Vane et al. 1990, Vanhoutte. 1989).

Arterial endothelial dysfunction is a key, early, and potentially reversible step in the process of atherogenesis and is characterized by impaired NO bioavailability (Berliner et al. 1995, Healy. 1990, Ross. 1993). Dysfunction of endothelial cells causes impaired vasomotor responses to numerous neurohumoral stimuli which may lead to temporary myocardial ischemia, thrombosis, plaque rupture, and
myocardial infarction (Maseri et al. 1978). So far, many well-established conventional CVD risk components, such as hypercholesterolemia, smoking, hypertension, obesity, microalbuminuria, IR and T2DM have been associated with endothelial dysfunction (Anderson et al. 1995, Goodfellow et al. 1996, Koller. 2002, McVeigh et al. 1992, Monhart. 2011, Treasure et al. 1995, Westerbacka & Yki-Järvinen. 2002, I. L. Williams et al. 2002, S. B. Williams et al. 1996). The extent of endothelial dysfunction is related to the rate of progression of atherosclerosis and CVD events (Schächinger et al. 2000, Widlansky et al. 2003). Therefore, arterial endothelial function is of significance, not only in determining predisposition to atherosclerotic disease, but also in determining prognosis in clinically affected individuals (Lane et al. 2006).

Both intima and media calcifications are associated with increased arterial stiffness, leading to higher rates of morbidity and mortality (Wilson et al. 2001), but they alter arterial functions by different mechanisms (Briet et al. 2012). Intima plaque calcification induces arterial dysfunction resulting from narrowing of the arterial lumen, with ischemia affecting the tissues and organs downstream (O'Rourke. 1995), which is common in atherosclerosis (London & Drueke. 1997). In turn, media calcification does not extend into the arterial lumen in its typical pure form, and it is associated with arterial-hardening arteriosclerosis (Guerin et al. 2000). The first consequence of media sclerosis is increased systolic BP, resulting in elevated cardiac afterload and left ventricular hypertrophy. The second one is decreased diastolic BP and impaired coronary perfusion (O'Rourke. 1995). Apart from age, diabetes is one of the most common causes of medial vascular calcification (Tolle et al. 2015).

Large elastic arteries, such as the aorta and pulmonary trunk, have thick, highly developed tunica media, of which elastic fibers are the dominant component. Vessel wall compliance is dependent on the status of two major proteins: collagen and elastin (Zieman et al. 2005). Normally, there is a tightly regulated balance between synthesis and degradation of these two proteins. Anomalies occur in this regulatory system such as that which accrues from inflammatory change, where collagen is overproduced and elastin synthesis is undermined (Johnson et al. 2001). Such asymmetry contributes to arterial stiffening. In addition, increased luminal pressure such as in hypertension also tends to favor collagen production at the expense of elastin (C. Xu et al. 2000). Generally, vascular stiffening occurs as a consequence of a complex interplay between several independent as well as interdependent factors. Figure 3 summarizes different mechanisms of arterial stiffening and locations in the arterial wall (Zieman et al. 2005).
Endothelial status and large artery stiffness can be measured in numerous ways using invasive or noninvasive methods in the coronary and peripheral circulation (Lane et al. 2006, Laurent et al. 2006). When considering noninvasive techniques, arterial compliance can be measured by using a radial artery tonometer (Laurent et al. 2006, McVeigh et al. 2002). However, carotid to femoral pulse wave velocity (PWV) has arisen as the gold standard to quantify arterial dysfunction. Further, values of central blood pressure (cBP) provide even more information concerning wave reflections (Laurent et al. 2006).

**Figure 3.** Different mechanisms of arterial stiffening and locations in the arterial wall according to Zieman et al. (2005). Further, perivascular fat related to abdominal obesity may independently increase arterial stiffness (Lim & Meigs. 2013).

![Diagram of arterial wall mechanisms](image)

AGE: advanced glycation end-product; MMP: matrix metalloproteinase; NaCl: sodium chloride; VSMC: vessel smooth-muscle cell

### 2.4.1 Arterial compliance

Systemic arterial compliance can be assessed noninvasively by using radial artery pulse wave analysis (Laurent et al. 2006, Nichols. 2005). The methodology gives measures of proximal capacitive compliance of large arteries (C1), including the aorta, and distal oscillatory compliance, which concerns endothelial function of the microvascular circulation or small arteries (C2) (Cohn. 1999, Laurent et al. 2006). This technique involves use of a modified Windkessel pulse-contour method, in
which the arterial system is likened to a fire-hose system: an air-filled dome, which softens flow pulsations generated by an occasionally working pump, is compared to the large arteries, the wide-bore hose acting as a pipeline, and the fire-hose nozzle is assimilated with the peripheral arterioles (Cohn et al. 1995, Nichols & O’Rourke. 2005, Nichols & McDonald. 1972).

In practice, the equipment automatically records arterial pulse waves at the level of the radial artery and identifies the reflections in diastole as a decaying sinusoidal wave (Cohn et al. 1995, Finkelstein et al. 1988, McVeigh et al. 1999, McVeigh. 2003). The higher the arterial compliance, the more elastic the wall of the vessel is considered to be. Further, when there is a reduction in compliance, mean BP usually increases. However, due to higher pressure oscillations, there seems to be a disproportionate increase in systolic BP and only a minor change in diastolic BP (Nichols & McDonald. 1972). By relying on numerous theoretical estimations following direct measurement of one peripheral and yet often distal parameter, there are some practical and technical limitations in the clinical use of arterial compliance. Decreased values of arterial compliance indices have been observed to be associated with MetS (Ge et al. 2008) and increased CVD risk as estimated by using SCORE and FINRISK risk models (Pohjantähti-Maaroos et al. 2012). Further, arterial compliance has broader clinical importance as it is associated with the pathogenesis of some non-CV outcomes including a variety of cognitive deficits such as Alzheimer’s disease, cerebral white-matter lesions, and kidney dysfunction (Kalaria et al. 2012, Mikael et al. 2017, Mitchell. 2004).

2.4.2 Pulse wave velocity

At every heartbeat, a pulse wave is generated, which then travels along the arterial tree. As a result of heterogeneity caused by cellular, molecular, and histological variation of the arterial wall, the elastic qualities of arteries change along the arterial system, with stiffer distal arteries and more elastic proximal ones (Bezie et al. 1998, Latham et al. 1985, Laurent et al. 2005, Mikael et al. 2017). In addition, the wall of the artery loses elasticity with aging, becoming more rigid (Kelly et al. 1989, Nichols. 2005, Vaitkevicius et al. 1993). Pulse wave velocity (PWV) is the speed at which the forward flow wave or pressure is transmitted from the aorta through the arterial bed (Cheung. 2010). In humans, PWV increases from 4–5 m/s in the ascending aorta to 5–6 m/s in the abdominal aorta, and further, to 8–9 m/s in the iliac and femoral arteries (Latham et al. 1985, Nichols & O’Rourke. 2005). PWV is
correlated inversely to arterial distensibility. In other words, the faster the PWV, the stiffer the artery. By providing a measure of mean stiffness of an arterial segment, PWV may provide a good reflection of overall vascular health (Cheung. 2010). The measurement of PWV is frequently accepted to be a robust, reproducible and straightforward non-invasive technique to assess arterial stiffness (Laurent et al. 2006). Furthermore, increased PWV is a powerful predictor of CVD events and mortality. According to a review written by Vlachopoulos et al. (2010), an increase in PWV of 1 m/s is correlated to a 14–15% increase in CVD events and mortality, as well as all-cause mortality (Vlachopoulos et al. 2010).

PWV is most often determined using the foot-to-foot velocity technique from diverse waveforms, which are commonly obtained transcutaneously at the right common carotid artery as well as the right femoral artery, and the time delay (Dt or transit time) is measured between the feet of the two waveforms (Laurent et al. 2006). The foot of the pulse wave seems to be relatively unaffected by wave reflections, and it is determined at the end of diastole, when the steep rise of the wavefront begins (Cheung. 2010, Laurent et al. 2006). The distance (D) along which the pulse travels is usually estimated by direct superficial measurement between the two pressure transducers or other devices used to register the pulse. Recording of the pulse waves at these two sites can be carried out simultaneously or by gating separate recordings to the R wave of the electrocardiogram, the first upward deflection after the P wave (Cheung. 2010). PWV is calculated as D (meters) divided by Dt (seconds). This so called carotid–femoral PWV is a direct measurement, and it fits the widely accepted propagative model of the arterial tree (Laurent et al. 2006).

There are several ways to register arterial pulse waves noninvasively, including using an oscillometric device, pressure-sensitive transducers, whole-body impedance cardiography, applanation tonometry, photoplethysmography, Doppler ultrasonography, and magnetic resonance imaging (Asmar et al. 1995, Cortez-Cooper et al. 2003, Kontis & Gosling. 1989, Loukogeorgakis et al. 2002, Mohiaddin et al. 1993, Wilenius et al. 2016, Wilkinson et al. 1998, Wright et al. 1990). Regardless of the method used, a possible source of error when measuring arterial pulse waves noninvasively is the necessity to use the nearest superficial arteries as a surrogate site for inaccessible central arteries as well as approximation of the actual D between recording sites by using surface measurements. The shorter the D between two recording sites, the greater the absolute error in determining Dt. Some investigators suggest either using the total D between the carotid and femoral sites of measurement or subtracting the distance from the carotid location.
to the sternal notch from the total D, or subtracting the distance from the carotid location to the sternal notch from the distance between the sternal notch and the femoral site of measurement (Van Bortel et al. 2002, van der Heijden-Spek et al. 2000). Despite these limitations, carotid–femoral PWV is definitely a gold standard method, and probably the most widely used for assessment of arterial stiffness (Cheung, 2010, Laurent et al. 2006).

2.4.3 Central blood pressure

Hypertension – a major risk feature of a variety of CV diseases – is commonly diagnosed by measuring BP at the brachial artery (Papaioannou et al. 2009). The prognostic value of brachial BP is well known (Agabiti-Rosei et al. 2007). However, such a measurement may exactly determine diastolic BP, but does not accurately reflect systolic BP. The BP waveform is distorted when travelling outward from the heart as a result of the presence of wave reflections from the peripheral arteries. Because of this aberration, brachial BP provides an inaccurate measure of central aortic systolic pressure (Papaioannou et al. 2009).

Vital organs are exposed to central rather than brachial BP (Kostapanos et al. 2016). Central BP (cBP) represents the true load imposed on the brain, heart and kidneys, and the central blood flow influences the local flow into these vital organs. An elevation of cBP has a direct adverse impact on the target organ and, thus, the prognosis of CVD in individuals with hypertension (Hashimoto. 2014). Among the different groups of antihypertensive drugs, beta-blockers appear to lower cBP less than brachial BP (Kostapanos et al. 2016). This difference may explain the decreased efficacy of beta-blockers in the prevention of CVD outcomes compared with the other classes of antihypertensive drugs, which lower central and brachial BP to a similar extent. Nevertheless, this differential effect might not be relevant to the newer beta-blockers with vasodilating properties (Kostapanos et al. 2016).

Systolic cBP is an important factor determining cardiac function and work, while diastolic cBP may determine coronary flow (Papaioannou et al. 2009). Today, cBP can be estimated noninvasively from peripheral pressure pulses through the use of several devices (Kostapanos et al. 2016, Miyashita. 2012). Accurate peripheral pressure pulse recording has been made possible by the introduction of arterial applanation tonometry, for which the radial artery may be the optimal site. In terms of objectivity and reproducibility, an automated tonometry device utilizing a sensor array is preferable. Calibration of a peripheral pressure waveform carries
unsolved problems for any estimation method. However, if central and peripheral pressure calibrations are equivalent, two major methods to estimate cBP – based on generalized pressure transfer function or radial late systolic pressure – may be comparable in their preciseness of cBP estimation (Miyashita. 2012).

Although values of cBP are indirect surrogate measures of arterial stiffness, they provide further information concerning pulse wave reflections (Nichols. 2005). Considerable evidence suggests that noninvasively determined cBP is pathophysiologically more relevant and a better predictor of end-organ damage than peripheral pressure (Kostapanos et al. 2016, Nelson et al. 2010, B. Williams et al. 2006). Furthermore, cBP also correlates with CVD risk in apparently healthy individuals (Agabiti-Rosei et al. 2007).
3 AIMS OF THE STUDY

The aim of this work was to study non-traditional biomarkers of CVD risk factors and arterial stiffness 2–6 years after pregnancy with and without gestational diabetes in order to elucidate the higher CVD risk in women with previous GDM. Another aim was to examine the effect of obesity on the results. Moreover, we wanted to assess the utility of MetS diagnosis when estimating individual CVD risk.

The specific aims of the study were to

1. determine the prevalence of MetS after previous GDM (I).

2. examine whether oxLDL, HOMA-IR or cBP differ between women with and without previous GDM (II).

3. investigate possible differences in the serum concentrations of hsCRP, MMP-8, -9 and TIMP-1, and in the measures of arterial stiffness after pregnancy complicated by GDM compared with normoglycemic pregnancy (III).

4. study the influence of obesity on the results (I–III).

5. assess the utility of MetS diagnosis when estimating individual CVD risk by evaluating the differences in arterial stiffness and CVD risk features between individually paired fertile women with and without MetS (IV).
4 SUBJECTS AND METHODS

4.1 Subjects and study design

This thesis consists of four substudies, referred to as I–IV in the text. All the examinations were performed at Kanta-Häme Central Hospital and Linnan Klinikka, Hämeenlinna, Finland. Both recruitment and examinations were carried out between August 2011 and July 2014 (I–IV).

Studies I–III were hospital-based studies of two cohorts. In these follow-up studies of 240 women, all of whom had undergone a 75-g OGTT during the index pregnancy, a total of 120 women with a history of GDM during the index pregnancy were compared with 120 age-matched women with normal glucose metabolism during pregnancy. All the participants were of Caucasian origin, and they had delivered 2–6 years earlier at Kanta-Häme Central Hospital, Finland, i.e. after the publication of Finnish Current Guidelines for screening GDM (I–III) (Gestational diabetes. Current Care Guidelines. 2013). GDM was defined as any pathological value in a 2-h 75-g OGTT during pregnancy (venous plasma glucose ≥ 5.3 mmol/L when fasting, ≥ 10.0 mmol/L at 1 h or ≥ 8.6 mmol/L at 2 h). The diagnostic criteria of GDM were the same as in Finnish Current Guidelines, which were published in 2008 and updated in 2013 without any change in the diagnostic criteria of GDM (Gestational diabetes. Current Care Guidelines. 2013). The electronic database of Kanta-Häme Central Hospital was used to pick up the cases and controls (Figure 4).

In summary, inclusion and exclusion criteria were as follows: singleton index pregnancy and delivery 2–6 years before participating in the follow-up study; GDM cohort: GDM defined as a pathological value in the 75-g OGTT according to Finnish Guidelines during the pregnancy (see above) (Gestational diabetes. Current Care Guidelines. 2013); Control cohort: normal OGTT results during the index pregnancy, no GDM in earlier pregnancy/pregnancies, and birth weight of the newborn < 4.5 kg. Women were also excluded if they had suspected or verified endocrine or malignant disease, diagnosed T1DM or T2DM before the index pregnancy, substance abuse or treatment, a known clinical history of psychiatric
illness or if they were pregnant at time of the study. Controls without GDM were excluded if they had been diagnosed with GDM in earlier pregnancy (I–III).

**Figure 4.** Flow chart describing the recruitment of two cohorts in Studies I–III. Further, under the dashed line it illustrates the division of four subgroups in Studies II & III. In Finland, GDM screening using a 75-g OGTT is offered to all pregnant women except those who are at the lowest risk: primiparous women < 25 years old, BMI ≤ 25 kg/m² and no known history of DM in first-degree relatives, or multiparous women < 40 years old, no GDM in previous pregnancy or pregnancies and BMI ≤ 25 kg/m² before the current pregnancy (Gestational diabetes. Current Care Guidelines. 2013). During the study period, we found 726 GDM women from the database of Kanta-Häme Central Hospital. GDM participants (n = 120) were selected randomly from the hospital database, which included both diet- and drug-treated gravidas with GDM. The BMI used in subgroup analyses was measured during the follow-up study.
During the study period, 42.5% of parturients had undergone OGTT screening for GDM in Kanta-Häme Central Hospital, meaning that 57.5% of pregnant women at that time were at the lowest risk and thus excluded from our study (I–III).

Power analyses were conducted to estimate the required number of participants. Concerning continuous variables, we worked on a difference of 10% with a standard deviation of 25% (Cohen’s d = 0.40). Regarding the presentation of MetS the expected proportions were 10% and 25%. When the significance level was set at 5% and power at 80%, the estimated numbers of participants as regards continuous and categorical variables were 99 and 100 in both groups, respectively (I–III).

Metabolic syndrome (MetS) was defined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) for women as the presence of at least three of the following five criteria (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002): 1) WC > 88 cm; 2) serum TGs ≥ 1.7 mmol/L; 3) serum HDL-C < 1.3 mmol/L; 4) BP ≥ 130/85 mmHg; 5) plasma glucose level ≥ 6.1 mmol/L, or DM. Further, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or T2DM were defined in a 2-h 75-g OGTT as follows: IFG: venous plasma glucose 6.1–6.9 mmol/L when fasting; IGT: venous plasma glucose 7.8–11.0 mmol/L at 2 h; T2DM: venous plasma glucose ≥ 7.0 mmol/L when fasting or > 11.0 at 2 h (Diabetes. Current Care Guidelines. 2018, WHO. 1999).

To study the effect of obesity on the results, the whole study population was divided into four subgroups according to BMI and previous GDM. In Study I, the whole study group of 240 women was divided into two halves according to median BMI, which was 27 kg/m². The BMI cut-off of 27 kg/m² represents the average BMI of among Finnish women relatively well (26.8 kg/m² according to the FINRISK 2012 Study (Borodulin et al. 2014)). In medical investigations of obesity, agencies have used a BMI cut-off point of 30 kg/m², but also 27 kg/m² with comorbidity (Colman. 2012). In Studies II and III, obesity was classified according to the WHO recommendation as BMI of ≥ 30 kg/m² (Report of a WHO consultation. 2000).

In (cross-sectional) Study IV, concerning the utility of MetS diagnosis when estimating individual CVD risk, 27 women with MetS were included from a total of 240 participants in the original study population. Every woman with MetS was compared with an individually paired counterpart without the syndrome. To avoid
the confounding effects of well-known CVD risk factors, the counterparts without MetS were matched according to age, previous GDM status, and serum concentrations of LDL-C and TC (IV) (Figure 5). Further, there was no significant difference in smoking history between the paired study groups.

Figure 5. Flow chart illustrating the study population in the cross-sectional Study IV. Besides GDM status, the matching parameters between MetS women and individually paired counterparts without the syndrome were age and serum concentrations of both TC and LDL-C. There was no significant difference in the proportion of current smokers between the paired study groups.

4.2 Methods

4.2.1 Individual interviews

Information on each participant’s medical history, CVD in the family, dietary habits, smoking, alcohol consumption, physical activity and lifetime weight loss was collected during a standardized interview. More closely, dietary habits were evaluated by inquiring about the consumption of meat, fish, sausage, berries, milk or low-fat milk products, sweets and sweet baked goods, butter, and margarine. Alcohol consumption was calculated as g/day according to the quantity of ethanol in different beverages such as beer, cider, wine or other alcoholic drinks, and the frequency of each beverage consumption. The participants were also asked about their average times, durations, types and intensity levels (four predetermined choices) of physical exercise per week. Further, the participants were interviewed as
regards their history of trauma or infectious diseases during the month before follow-up examinations. Information on the index pregnancy, delivery and perinatal outcome was collected using the hospital database. Smoking status was categorized as current, former or never. Lifetime tobacco exposure was calculated as pack-years by multiplying smoking years by the average number of packs smoked daily. One pack-year was defined as twenty cigarettes smoked every day for one year (Saquib et al. 2013). Initially successful weight loss followed by weight regain, i.e. so called “yo-yo” dieting or weight cycling, is associated with body-weight excess and abdominal fat accumulation (Cereda et al. 2011). To analyze that, total lifetime weight loss was estimated by adding together kilograms lost during every previous intentional weight-loss period.

4.2.2 Physical examinations

Weight (kg) and height (cm) of the participants were measured according to general recommendations. Brachial BP and heart rate were recorded by using an automatic electronic BP meter after at least ten minutes of rest in a semi-sitting position. At least three consecutive measurements of BP (with resolution of 1 mmHg) were performed to achieve average results for every woman. Pulse pressure (PP) was calculated as systolic minus diastolic BP. Waist circumference (WC) was measured midway between the lowest rib and the iliac crest at the midaxillar line to the nearest centimeter. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m²).

4.2.3 Clinical chemistry and immunoassays

Basic blood cell count (Laboratory of Linnan Klinikka, Hämeenlinna, Finland) and serum levels of creatinine, alanine transaminase (ALAT), fP-Gluc, TC, HDL-C, LDL-C and TGs, and the urinary albumin to creatinine ratio, as well as plasma fibrinogen, were analyzed according to validated methods. Direct analyses of TC, HDL-C, LDL-C and TGs were carried out by using commercial reagents from Beckman Coulter (Brea, CA, USA). Non-HDL-C was calculated by subtracting HDL-C from TC (Orakzai et al. 2009). Analyses of ALAT (IFCC method), creatinine (Jaffé method), plasma glucose (hexokinase method), and glycosylated hemoglobin (HbA1c) were carried out by using commercial reagents from Beckman Coulter, with an Olympus AU640 analyzer, and analyses of fibrinogen
(Clauss method) by using Siemens BCS XP equipment. Plasma insulin levels were measured by electrochemiluminescence immunoassay (ECLIA) (Roche Cobas, Basel, Switzerland). Serum concentrations of hsCRP were analyzed according to validated immunonephelometric (United Medix Laboratories Ltd., Espoo, Finland) and immunoturbidimetric methods (Chenillot et al. 2000, Sanchez et al. 2002). All the samples were collected into EDTA, lithium-heparin gel, or sodium fluoride tubes according to laboratory instructions after at least 12 hours of fasting and, after cold centrifugation, samples were stored at -80°C until analyzed. Clinical chemistry and immunoassays were carried out by VITA Healthcare Services Ltd., Vita Laboratory, Helsinki, Finland, if not mentioned otherwise.

4.2.3.1 Oxidized low-density lipoprotein

Plasma concentrations of oxLDL were determined by using a validated enzyme-linked immunosorbertent assay (ELISA) (Mercodia AB, Uppsala, Sweden). The reagents include the same monoclonal antibody (4E6) as originally described by Holvoet et al. (Holvoet et al. 1998, Holvoet et al. 2001). Plasma samples were diluted with sample buffer in two steps to gain a final dilution 1/6561. Of each calibrator, control and diluted sample, 25 µL were pipetted into wells containing mouse monoclonal anti-oxidized LDL. Assay buffer (100 µL) was added to each well, after which the plate was incubated on a plate shaker for 120 minutes at room temperature. After the incubation period, the samples were washed six times with an automatic washer before 100 µL peroxidase-conjugated mouse monoclonal anti- apoB was added to the wells. After 60-minute incubation at room temperature, the samples were washed again and the bound conjugate was detected by reaction with 200 µL 3.3', 5.5'-tetramethylbenzidine. The reaction was stopped by adding 50 µL H₂SO₄ at 0.5 mmol/L and the colorimetric endpoint was read spectrophotometrically at 450 nm. An Evolis ELISA analyzer (Bio-Rad, Marnes-la-Coquette, France) was used to run the assays. Analysis of plasma levels of oxLDL is based on the standards included in each separate assay. The results were expressed as units per liter (U/L). The total coefficient of variation of the assay including both inter-assay and intra-assay variability was 8.5% (II).
4.2.3.2 Matrix metalloproteinase-8, and -9 and tissue inhibitor of metalloproteinase-1

Concentrations of MMP-8 were measured by a time-resolved immunofluorometric assay (IFMA) (Medix Biochemica, Espoo, Finland). Monoclonal MMP-8-specific antibodies 8708 and 8706 were used as a catching antibody and a tracer antibody, respectively. The tracer antibody was labeled with europium chelate. The assay buffer contained 20 mM Tris-HCl, pH 7.5, 0.5 µM NaCl, 5 mM CaCl$_2$, 50 µM ZnCl$_2$, 0.5% bovine serum albumin, 0.05% sodium azide, and diethylenetriaminepenta-acetic acid (DTPA) at 20 mg/L. Samples were diluted in assay buffer and incubated for 1 h, followed by incubation for 1 h with tracer antibody. Enhancement solution was added, and after 5 min fluorescence was measured using a 1234 Delfia Research Fluorometer (Wallac, Turku, Finland) (Hanemaaijer et al. 1997). The coefficient of variation of inter-assay for MMP-8 was 4.1%, and that of intra-assay 2.5% (III).

Serum levels of MMP-9 and TIMP-1 (the Scientific Laboratory of the Department of Oral and Maxillofacial Diseases, Helsinki University and University Hospital, Finland) were determined by using commercially available ELISA kits. Biotrak ELISA systems kits for MMP-9 (Amersham Biosciences, GE Healthcare, Buckinghamshire, UK) were used according to the manufacturer’s instructions. DuoSet ELISA development Systems kits for TIMP-1 (R&D Systems, Minneapolis, USA) were used correspondingly. All samples were analyzed in duplicate. According to the manufacturers the MMP-9 and TIMP-1 ELISAs detect active, pro-, complexed and fragmented forms of the analytes. The secondary antibody in each kit was conjugated with horseradish peroxidase, and tetramethylbenzidine was used as a substrate. Absorbance was measured at 450 nm using Labsystems Multiskan RC equipment (Thermo Bioanalysis Corporation, Santa FE, USA). The levels of MMP-8 and -9 and TIMP-1 were expressed as ng per mL, and for calculation of MMP-8 and -9/TIMP-1 molar ratios the levels were converted to mol per L (Rautelin et al. 2009). The coefficient of variation of inter-assay for MMP-9 was 8.8%, and for TIMP-1, 13.1%; and those of intra-assay for MMP-9 was 5.1% and for TIMP-1, 10.1% (III).

4.2.4 The homeostasis model of insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) index is based on single measurements of glucose and insulin in the blood and is commonly
used as a parameter reflecting the severity of IR (Monzillo & Hamdy. 2003). HOMA-IR was calculated by multiplying the fasting plasma insulin (fP-Insu) level by that of fasting plasma glucose (fP-Gluc), and dividing by 22.5 \[\frac{fP-\text{Insu} \text{ (mU/L)} \times fP-\text{Gluc} \text{ (mmol/L)}}{22.5}\] (D. R. Matthews et al. 1985) (II).

4.2.5 Non-invasive measurements of arterial function

Altogether, four experienced nurses performed the non-invasive measures of arterial function. Participants were asked to refrain from eating, having caffinated drinks, smoking and taking medication for 12 hours, and drinking alcohol for two days prior to measurement. All the measurements were done after the subject had had at least ten minutes of rest in a semi-sitting position. At least three consecutive recordings of all non-invasive measurements were performed to achieve average results for every woman (II–IV).

4.2.5.1 Arterial compliance

Radial artery pulse waves were recorded non-invasively with an arterial tonometer (HDI/PulseWaveTMCR-2000, Hypertension Diagnostics, Inc., Eagan, Minnesota, USA) and the procedure involves the use of a modified Windkessel pulse-contour method (Cohn et al. 1995). Blood volume inertia and systemic vascular resistance were used to analyze arterial compliance. The capacitive compliance of large arteries (C1), including the aorta, and the endothelial function of small arteries (C2) were automatically assessed as a mean of the five most similar pulse waves appearing during thirty seconds of measurement (III & IV).

4.2.5.2 Pulse wave velocity

Pulse wave velocity (PWV) was determined using the foot-to-foot velocity method from carotid and femoral waveforms by employing a SphygmoCor® device (AtCor Medical, Sydney, Australia) (Figure 6). Transcutaneous readings were gained at the right common carotid artery and the right femoral artery with the subjects in a supine position with direct-contact pulse sensors. The time delay (Dt or transit time) of the two waveforms was registered, and the distance (D) between carotid and femoral recording sites was obtained by subtracting the carotid measurement.
site to sternal notch distance from the sternal notch to the femoral measurement site distance. PWV was calculated as \( \frac{D}{Dt} \) (m/s) (Agabiti-Rosei et al. 2007, Laurent et al. 2006). Only measurements that met the automatic quality control cut-off were used in the final analysis (III & IV).

**Figure 6.** PWV measured using the foot-to-foot velocity method from the waveforms of carotid and femoral arteries.

4.2.5.3 Central blood pressure

Central blood pressure (cBP) was estimated non-invasively from the radial artery pulse wave by way of a SphygmoCor® device (AtCor Medical, Sydney, Australia), which uses radial pulse and a validated generalized transfer function to estimate central pressures from brachial BP and the peripheral pulse waves (Agabiti-Rosei et al. 2007) (II & IV).
4.3 Statistical Analyses

The data were analyzed by using IBM® SPSS® Statistics Version 22 (copyright 2013) and 23 software (copyright 2015). Variables were tested for normality by way of Shapiro–Wilk or Kolmogorov–Smirnov tests, as appropriate. Data are presented as mean ± standard deviation (SD) if not mentioned otherwise. A two-tailed probability value of < 0.05 was considered significant (I–IV).

In Studies I–III, differences in continuous variables between GDM and control cohorts were studied by using Student's t-test in cases of normality and by the Mann–Whitney U-test in cases of non-normality. Categorical data are presented as percentages and were compared by using the chi-square test. The correlations between different variables were tested by Pearson’s or Spearman’s correlation analysis, as appropriate.

The clinical characteristics of the four subgroups made according to BMI and previous GDM were compared by way of one-way ANOVA in cases of normality and by using the Kruskal–Wallis test in cases of non-normality. Post hoc analyses were performed by using Fisher's least significant difference method or, in order to correct for multiple testing, by using a conservative Bonferroni correction factor.

Univariate linear regression analyses were conducted to find possible associations with clinically relevant covariates. Multiple linear regression analyses were carried out to examine whether simple associations were changed after adjustment for potential confounders. Finally, stepwise multiple linear regression analyses were done to find relevant covariates in final models. F-statistics was used to optimize the sequential variable selection procedure.

In Study IV, differences in continuous variables between MetS participants and individually paired counterparts without the syndrome were studied by using paired t-tests in cases of normality and by using Wilcoxon’s test in cases of non-normality. Differences in binomial outcomes between the two paired study groups were tested by using McNemar’s test. The Hodges–Lehmann estimator was used for assessing differences in medians (with 95% CIs) between MetS participants and their matched controls.

4.4 Ethical considerations

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (World Medical Association Inc. 2009), and the protocol
was approved by the Ethics Committee of Kanta-Häme Hospital District (reference number 521/2010; date of approval 21.12.2010). Each participant was given both oral and written information on the study before she signed an informed consent document. All data were analyzed anonymously.
5 RESULTS

5.1 Follow-up study of two cohorts: women with previous gestational diabetes mellitus and controls (I–III)

Basic information on the index pregnancy and clinical characteristics in the follow-up study in the GDM and control groups are presented in Table 6. Twenty-three of the 120 women were primiparous in both cohorts. A total of 25 GDM participants had antihyperglycemic medication during their pregnancies (insulin, n = 24; metformin, n = 1), while the rest (n = 95) of the women in the GDM group had only dietary therapy. Almost thirty percent (29.9%, n = 29/97) of the multiparous GDM women had had GDM in an earlier pregnancy. Accumulation of pregnancy-induced hypertensive disorders was more common in GDM pregnancies. In the GDM group, induction of labor was more common than in the control group, but no difference was found in the rate of cesarean section. Regarding perinatal outcome, base excess in umbilical venous blood tended to be higher in controls, but otherwise perinatal outcomes did not differ between the study cohorts.

Current Finnish guidelines recommend OGTT screening six to twelve weeks after delivery in cases of medicated GDM during pregnancy, and one year after delivery in diet-treated GDM during pregnancy (Gestational diabetes. Current Care Guidelines. 2013). Despite that, only 41 of the 120 GDM women (34.2%) had undergone an OGTT after delivery. Of these, 39.0% expressed glucose intolerance as follows: 17.1% had IFG, 14.6% had IGT and 7.3% had diabetes. Twenty-five of the 41 cases had normal results in postpartum OGTT screening (Table 6).

In both study cohorts, the mean time to follow-up was 3.7 years. During the follow-up study, women were aged 35.8 ± 4.5 (range 25 to 46) in the two groups. There were no differences in family history of coronary heart disease (GDM 16.7% vs. controls 19.2%, p = 0.737) or DM (GDM 26.7% vs. controls 22.5%, p = 0.549), but a family history of cerebrovascular disease (GDM 12.5% vs. controls 4.2%, p = 0.033) differed significantly between the women with and without previous GDM. There were no differences in permanent medication for any chronic disease (GDM
35.8% vs. controls 29.2%, \( p = 0.335 \) or use of hormonal contraception (GDM 49.2% vs. controls 44.2%, \( p = 0.518 \)) between the cohorts.

Table 6. Baseline information on the index pregnancy in GDM and control cohorts. Data on pregestational BMI were available from 110 GDM and 108 control women, and data on smoking during the pregnancy in 99 GDM and 102 control women.

<table>
<thead>
<tr>
<th></th>
<th>GDM ( n = 120 )</th>
<th>Controls ( n = 120 )</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td></td>
</tr>
<tr>
<td><strong>Index pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregestational BMI, kg/m(^2)</td>
<td>28.3 5.4</td>
<td>27.5 5.3</td>
<td>0.215</td>
</tr>
<tr>
<td>Smoking during the pregnancy, n (%)</td>
<td>10 (8.3%)</td>
<td>5 (4.2%)</td>
<td>0.187</td>
</tr>
<tr>
<td>Primiparous, n (%)</td>
<td>23 (19.2%)</td>
<td>23 (19.2%)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>75-g OGTT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-h, mmol/L</td>
<td>5.4 0.5</td>
<td>4.7 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1-h, mmol/L</td>
<td>9.5 2.3</td>
<td>7.1 1.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2-h, mmol/L</td>
<td>7.7 2.0</td>
<td>5.8 1.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Therapy of GDM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, n (%)</td>
<td>24 (20.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin, n (%)</td>
<td>1 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet only, n (%)</td>
<td>95 (79.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy-induced hypertension, n (%)</td>
<td>19 (15.8%)</td>
<td>8 (6.7%)</td>
<td>0.038</td>
</tr>
<tr>
<td>Induction of labor, n (%)</td>
<td>42 (35.0%)</td>
<td>26 (21.7%)</td>
<td>0.031</td>
</tr>
<tr>
<td>Rate of cesarean section, n (%)</td>
<td>29 (24.2%)</td>
<td>21 (17.5%)</td>
<td>0.266</td>
</tr>
<tr>
<td><strong>Perinatal outcome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, days</td>
<td>277.1 9.5</td>
<td>278.8 10.4</td>
<td>0.112</td>
</tr>
<tr>
<td>Birth weight of the child, g</td>
<td>3633 519</td>
<td>3540 471</td>
<td>0.107</td>
</tr>
<tr>
<td>Apgar at one minute</td>
<td>8.6 1.2</td>
<td>8.7 1.4</td>
<td>0.146</td>
</tr>
<tr>
<td>Apgar at five minutes</td>
<td>9.3 0.8</td>
<td>9.3 0.8</td>
<td>0.657</td>
</tr>
<tr>
<td>UA-pH</td>
<td>7.29 0.1</td>
<td>7.28 0.1</td>
<td>0.059</td>
</tr>
<tr>
<td>UA-BE</td>
<td>2.4 2.4</td>
<td>3.0 2.6</td>
<td>0.054</td>
</tr>
<tr>
<td>UV-pH</td>
<td>7.35 0.1</td>
<td>7.35 0.1</td>
<td>0.409</td>
</tr>
<tr>
<td>UV-BE</td>
<td>2.8 2.4</td>
<td>3.3 2.3</td>
<td>0.045</td>
</tr>
<tr>
<td><strong>OGTT screening after delivery, n (%)</strong></td>
<td>41 (34.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFG, n (%)</td>
<td>7 (17.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGT, n (%)</td>
<td>6 (14.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, n (%)</td>
<td>3 (7.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, n (%)</td>
<td>25 (61.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BE: base excess; BMI: body mass index; DM: diabetes mellitus; GDM: gestational diabetes mellitus; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; OGTT: oral glucose tolerance test; UA: umbilical artery; UV: umbilical vein
During the follow-up study, there were more current or former smokers in the GDM group than in the control group according to study interview data, and pack-years of smoking also differed significantly. The groups did not differ in alcohol intake, physical activity, or lifetime weight loss. The proportion of GDM women using margarine weekly was less than in the control group (GDM 53.3% vs. controls 67.5%; p = 0.034), but on the other hand the proportions of weekly use of butter did not differ between the groups (GDM 57.5% vs. controls 55.0%; p = 0.795). The percentage of GDM participants who consumed sweets and sweet baked goods weekly was smaller than in control women (GDM 79.2% vs. controls 92.5%; p = 0.005). Otherwise, no other differences were found in basic nutrition habits between the groups.

Of the whole study population, one woman in the GDM group did not take part in laboratory examinations. Basic laboratory results concerning the women with and without previous GDM are presented in Table 7. Concentrations of leukocytes (p = 0.008), hemoglobin (p = 0.001) and creatinine (p = 0.048) were higher among GDM women than in controls. The urinary albumin/creatinine ratio (U-AlbCre) tended to be higher among GDM women, but the difference was nonsignificant (p = 0.070).
Table 7. Clinical characteristics and laboratory findings in the follow-up study in the GDM and control cohorts.

<table>
<thead>
<tr>
<th></th>
<th>GDM n = 120</th>
<th>Controls n = 120</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td></td>
</tr>
<tr>
<td>Follow-up study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average time since delivery (years)</td>
<td>3.7  1.0</td>
<td>3.7  0.9</td>
<td>0.818</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.8 4.4</td>
<td>35.9 4.6</td>
<td>0.854</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td>0.018</td>
</tr>
<tr>
<td>Current, n (%)</td>
<td>24  (20.0%)</td>
<td>12  (10.0%)</td>
<td></td>
</tr>
<tr>
<td>Former, n (%)</td>
<td>45  (37.5%)</td>
<td>37  (30.8%)</td>
<td></td>
</tr>
<tr>
<td>Never, n (%)</td>
<td>51  (42.5%)</td>
<td>71  (59.2%)</td>
<td></td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>3.8  6.0</td>
<td>2.4  4.6</td>
<td>0.012</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3  5.0</td>
<td>27.5  5.4</td>
<td>0.069</td>
</tr>
<tr>
<td>WC, cm</td>
<td>96.8  13.0</td>
<td>92.5  12.6</td>
<td>0.009</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>122.4  12.5</td>
<td>119.0  11.5</td>
<td>0.034</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73.5  9.0</td>
<td>71.8  8.7</td>
<td>0.176</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>65.9  9.1</td>
<td>63.8  9.6</td>
<td>0.017</td>
</tr>
<tr>
<td>Clinical chemistry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes, 10⁹/L</td>
<td>5.8  1.6</td>
<td>5.2  1.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>133.2  9.3</td>
<td>128.6  12.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelets, 10⁹/L</td>
<td>241.9  58.2</td>
<td>244.0  52.5</td>
<td>0.692</td>
</tr>
<tr>
<td>ALAT, U/L</td>
<td>22.8  17.4</td>
<td>19.7  10.5</td>
<td>0.116</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>66.6  7.7</td>
<td>64.5  7.8</td>
<td>0.048</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.4  0.9</td>
<td>3.2  1.0</td>
<td>0.096</td>
</tr>
<tr>
<td>U-AlbCre, mg/mmol</td>
<td>0.67  0.5</td>
<td>0.57  0.3</td>
<td>0.070</td>
</tr>
</tbody>
</table>

ALAT: alanine transaminase; BMI: body mass index; BP: blood pressure; GDM: gestational diabetes mellitus; OGTT: oral glucose tolerance test; U-AlbCre: urinary albumin/creatinine ratio; WC: waist circumference

5.2 Risk factors of cardiovascular disease after gestational diabetes mellitus (I–III)

5.2.1 Metabolic syndrome (I)

After pregnancy complicated by GDM, the women fulfilled the criteria of MetS 2.4-fold more often than did the controls. In the whole study population, the prevalence of MetS was 11.3%, while the prevalence in the GDM cohort was
15.8% and in the controls, 6.7% (p = 0.039). Defined by NCEP ATP III, the numbers of participants (%) with separate variables of MetS syndrome are presented in Table 8. Three women in the GDM group and five in the control group had permanent antihypertensive medication. Only one woman in the GDM cohort had treatment for lipid abnormality.

Previous GDM (OR 2.63, 95% CI 1.11–6.28; p = 0.029) was also associated with an increased risk of MetS in univariate logistic regression analysis, along with greater lifetime weight loss (OR 1.02, 95% CI 1.00–1.03; p = 0.013), higher BMI values calculated per one BMI unit (OR 1.24, 95% CI 1.14–1.35; p < 0.001) and higher levels of TC (OR 1.98, 95% CI 1.26–3.10; p = 0.003). Further, multivariate analysis indicated that previous GDM (OR 2.83, 95% CI 1.05–7.63; p = 0.040), higher serum concentrations of TC per one mmol/L (OR 1.68, 95% CI 1.01–2.79; p = 0.046) and higher BMI values calculated per one BMI unit (OR 1.24, 95% CI 1.13–1.36; p < 0.001) appeared to be associated with the manifestation of MetS.

Table 8. Prevalence of metabolic syndrome (MetS) and numbers of participants with separate variables of MetS defined by NCEP ATP III in a setting of two cohorts.

<table>
<thead>
<tr>
<th></th>
<th>GDM n = 120</th>
<th>Control n = 120</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetS, n (%)</td>
<td>19 (15.8%)</td>
<td>8 (6.7%)</td>
<td>0.039</td>
</tr>
<tr>
<td>WC &gt; 88 cm</td>
<td>89 (74.2%)</td>
<td>73 (60.8%)</td>
<td>0.038</td>
</tr>
<tr>
<td>TGs ≥ 1.7 mmol/L</td>
<td>12 (10.0%)</td>
<td>5 (4.2%)</td>
<td>0.084</td>
</tr>
<tr>
<td>HDL-C &lt; 1.3 mmol/L</td>
<td>23 (19.2%)</td>
<td>22 (18.3%)</td>
<td>0.870</td>
</tr>
<tr>
<td>BP ≥ 130/85 mmHg</td>
<td>35 (29.2%)</td>
<td>25 (20.8%)</td>
<td>0.179</td>
</tr>
<tr>
<td>fP-Gluc ≥ 6.1 mmol/L or DM</td>
<td>18 (15.0%)</td>
<td>4 (3.3%)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

BP: blood pressure; fP-Gluc: fasting plasma glucose; HDL-C: high-density lipoprotein cholesterol; DM: diabetes mellitus; GDM: gestational diabetes mellitus; MetS: metabolic syndrome; NCEP ATP III: National Cholesterol Education Program Adult Treatment Panel III; TGs: triglycerides; WC: waist circumference

5.2.2 Glucose metabolism and homeostasis model assessment of insulin resistance (I & II)

When women with previous GDM pregnancy were compared to women with previous normoglycemic pregnancy, there were significant differences in fasting plasma concentrations of glucose and HbA1c, but no difference in levels of fP-Insu. Further, HOMA-IR index values were significantly higher in the GDM
cohort. Variables of glucose metabolism in GDM women and controls are illustrated in Table 9.

When GDM women with medication (n = 25) were compared with those with diet therapy (n = 95) during the index pregnancy, there was a significant difference only in fP-Gluc (6.0 ± 1.0 vs. 5.5 ± 0.4 mmol/L, p = 0.003). When comparing drug-treated GDM women (n = 25), diet-treated GDM women (n = 95) and controls (n = 120), a significant difference was observed in HOMA-IR index values (p = 0.016). The HOMA-IR value among medicated GDM women was 1.6 ± 1.3, among diet-treated GDM women 1.2 ± 0.8 and among controls 1.1 ± 0.8 (p = 0.034 for controls vs. medicated GDM women; other comparisons were non-significant).

Table 9. Glucose metabolism and HOMA-IR in women with previous GDM and controls after normoglycemic pregnancy.

<table>
<thead>
<tr>
<th>Variable of glucose metabolism</th>
<th>GDM n = 119</th>
<th>Controls n = 120</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>fP-Gluc, mmol/L</td>
<td>5.61</td>
<td>0.70</td>
<td>5.26</td>
</tr>
<tr>
<td>fP-Insu, mU/L</td>
<td>5.21</td>
<td>3.63</td>
<td>4.63</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.30</td>
<td>0.91</td>
<td>1.09</td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>34.9</td>
<td>3.28</td>
<td>33.8</td>
</tr>
</tbody>
</table>

Table 9: Glucose metabolism and HOMA-IR in women with previous GDM and controls after normoglycemic pregnancy.

fP-Gluc: fasting plasma glucose; fP-Insu: fasting plasma insulin; GDM: gestational diabetes mellitus; HbA1C: glycosylated hemoglobin A1c; HOMA-IR: homeostasis model assessment of insulin resistance

According to the International Expert Committee (IEC), glycemic categories based on HbA1c cut-off points are as follows: normal, HbA1c < 42 mmol/mol; prediabetes, HbA1c ≥ 42 mmol/mol, but < 48 mmol/mol; and diabetes, HbA1c ≥ 48 mmol/mol (Gillett. 2009). In the current study population, one woman had DM and four had prediabetes in the GDM cohort, while all women in the control group were in the normal glycemic category according to their HbA1c levels (p = 0.076).

In multiple linear regression analysis, BMI was a significant determinant of the HOMA-IR index. However, previous GDM was not a crucial influencing factor of HOMA-IR in these analyses.
5.2.3 Lipids and oxidized low-density lipoprotein (I & II)

Concentrations of lipids and oxLDL in GDM women and controls are demonstrated in Table 10. Between the study cohorts, there was a significant difference only in serum levels of TGs. There were no differences in plasma concentrations of TC, HDL-C or LDL-C. Neither did oxLDL levels differ in women with GDM vs. controls. In multiple linear regression analysis, neither BMI nor previous GDM were associated with plasma levels of oxLDL.

Table 10. Lipids and oxLDL in GDM women and controls.

<table>
<thead>
<tr>
<th>Lipids</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean n = 119</td>
<td>Mean n = 120</td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.71</td>
<td>4.59</td>
<td>0.329</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.51</td>
<td>1.56</td>
<td>0.450</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.94</td>
<td>2.84</td>
<td>0.295</td>
</tr>
<tr>
<td>TGS, mmol/L</td>
<td>1.10</td>
<td>0.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>non-HDL-C, mmol/L</td>
<td>3.21</td>
<td>3.03</td>
<td>0.167</td>
</tr>
<tr>
<td>oxLDL, U/L</td>
<td>42.4</td>
<td>39.7</td>
<td>0.120</td>
</tr>
</tbody>
</table>

HDL-C: high-density lipoprotein cholesterol; GDM: gestational diabetes mellitus; LDL-C: low-density lipoprotein cholesterol; oxLDL: oxidized low-density lipoprotein; TC: total cholesterol; TGS: triglycerides

5.2.4 Low-grade inflammation (III)

During the previous month before follow-up laboratory examinations, no significant differences were found between the GDM and control cohorts in self-reported histories of infectious diseases or traumas. There was no difference in the levels of hsCRP between women with and without previous GDM, even when women affected by infectious diseases or traumas were excluded (data not shown). Serum concentrations of hsCRP were analyzed by both immunonephelometric and immunoturbidimetric methods, with the same results (data not shown). In multiple-adjusted analysis, only BMI was a significant determinant of hsCRP concentrations, but the model explained only 9.6% of hsCRP variation. Previous GDM did not explain current hsCRP levels.

After the index pregnancy, serum concentrations of TIMP-1 were significantly higher in GDM mothers compared with controls. However, no differences were
observed in the circulating levels of MMP-8 or MMP-9 between the study cohorts. Previous GDM, hsCRP and TC were important determinants of MMP-8 concentrations in stepwise multiple-adjusted analysis. Likewise, previous GDM, together with BMI and heart rate were associated with TIMP-1 levels in these analyses. Nevertheless, the model explained only 13.8% of MMP-8 and 6.7% of TIMP-1 variation. All determined variables of low-grade inflammation in the GDM and control cohorts are shown in Table 11.

Table 11. Variables of low-grade inflammation in GDM women and controls. Concentrations of hsCRP were analyzed by an immunonephelometric method.

<table>
<thead>
<tr>
<th>Variable of low-grade inflammation</th>
<th>GDM n = 119</th>
<th>Controls n = 120</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>2.51</td>
<td>3.69</td>
<td>2.50</td>
</tr>
<tr>
<td>MMP-8, ng/mL</td>
<td>27.8</td>
<td>16.1</td>
<td>32.8</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>384.3</td>
<td>143.5</td>
<td>392.2</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>102.8</td>
<td>29.7</td>
<td>94.6</td>
</tr>
</tbody>
</table>

hsCRP: high-sensitivity C-reactive protein; GDM: gestational diabetes mellitus; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase

5.3 Arterial function after gestational diabetes mellitus (II & III)

After GDM, PWV values were significantly higher than after normoglycemic pregnancy (Table 12). PWV was associated significantly with age (p < 0.001), BMI (p < 0.001), fP-Insu (p < 0.001), heart rate (p < 0.001), systolic BP (p < 0.001), TC (p < 0.001) and previous GDM (p = 0.009) in univariate linear regression analysis. In stepwise multiple-adjusted analysis, significant determinants of PWV values were systolic BP, age, insulin levels, previous GDM and time since the index pregnancy. These variables together explained 47.0% of PWV variation.

There was a nonsignificant difference in C1 values between the study groups. Further, no difference was observed in C2 values. In stepwise multiple linear regression analysis, systolic BP, heart rate, BMI and time since the index pregnancy were significant covariates explaining 52.4% of C1 values. Significant determinants of C2 values were systolic BP, heart rate, BMI, age and pack-years of smoking. These covariates explained 31.7% of C2 values. Differences in systolic and diastolic cBP did not reach statistical significance between the study groups. Neither did we find any difference in central mean pressure (90.7 ± 10.3 vs. 88.3 ± 9.5 mmHg; p =
0.089). All the non-invasive measurements of arterial function in the study cohorts are presented in Table 12.

Table 12. Variables of arterial stiffness in GDM women and controls.

<table>
<thead>
<tr>
<th>Determinant of arterial stiffness</th>
<th>GDM n = 120</th>
<th>Controls n = 120</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1, mL/mmHg×10</td>
<td>Mean 15.1</td>
<td>SD 3.51</td>
<td>Mean 15.9</td>
</tr>
<tr>
<td>C2, mL/mmHg×100</td>
<td>Mean 8.44</td>
<td>SD 3.08</td>
<td>Mean 8.60</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>Mean 6.44</td>
<td>SD 0.83</td>
<td>Mean 6.17</td>
</tr>
<tr>
<td>Systolic cBP, mmHg</td>
<td>Mean 110.6</td>
<td>SD 12.4</td>
<td>Mean 107.5</td>
</tr>
<tr>
<td>Diastolic cBP, mmHg</td>
<td>Mean 74.5</td>
<td>SD 9.11</td>
<td>Mean 72.7</td>
</tr>
</tbody>
</table>

cBP: central blood pressure; C1: large arterial compliance; C2: small arterial compliance; GDM: gestational diabetes mellitus; PWV: pulse wave velocity

5.4 Effect of obesity (I–III)

Both of the study groups, i.e. all 240 women, were included in subgroup analyses to investigate the effect of excess body weight and obesity on the primary results. In Study I, the whole study population of 240 women was divided into two halves according to median BMI, which was 27 kg/m². When using this cut-off point, there were 122 women in the “obese” group (BMI ≥ 27 kg/m²); 65 GDM and 57 control participants. The “non-obese” group (BMI < 27 kg/m²; n = 118) consisted of 55 GDM and 63 control participants. In Studies II and III, obesity was classified as BMI of ≥ 30 kg/m². Altogether, there were 75 women in the obese group (BMI ≥ 30 kg/m²); 43 GDM and 32 control participants. The non-obese group (BMI < 30 kg/m²; n=165) consisted of 77 GDM and 88 control participants. Regardless of the BMI cut-off point, there were differences in most of the basic clinical characteristics between these four subgroups, particularly between non-obese and obese subgroups. Results of subgroup analyses with a BMI cut-off of 30 kg/m² are shown in Table 13, while those with a BMI cut-off of 27 kg/m² are presented in Study I.
### Table 13. Results of follow-up study in four subgroups.

<table>
<thead>
<tr>
<th>Follow-up study</th>
<th>GDM</th>
<th>Control</th>
<th>Overall p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age, years</td>
<td>35.8</td>
<td>4.4</td>
<td>35.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.7</td>
<td>3.1</td>
<td>25.3</td>
</tr>
<tr>
<td>Pack years of smoking</td>
<td>4.8</td>
<td>8.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Weight loss during lifetime, kg</td>
<td>34.1</td>
<td>29.3</td>
<td>15.5</td>
</tr>
<tr>
<td>MetS, n (%)</td>
<td>11 (25.6%)</td>
<td>8 (10.4%)</td>
<td>7 (21.9%)</td>
</tr>
</tbody>
</table>

### Clinical chemistry and immunoassays

<table>
<thead>
<tr>
<th></th>
<th>GDM</th>
<th>Control</th>
<th>Overall p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG, mmol/L</td>
<td>5.7</td>
<td>0.4</td>
<td>5.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>34.8</td>
<td>2.4</td>
<td>34.9</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.87</td>
<td>0.88</td>
<td>4.83</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.45</td>
<td>0.29</td>
<td>1.54</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.10</td>
<td>0.08</td>
<td>2.86</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.29</td>
<td>0.75</td>
<td>1.00</td>
</tr>
<tr>
<td>non-HDL-C, mmol/L</td>
<td>3.34</td>
<td>1.02</td>
<td>3.09</td>
</tr>
<tr>
<td>oxLDL, U/L</td>
<td>44.8</td>
<td>14.6</td>
<td>41.3</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>3.3</td>
<td>3.7</td>
<td>2.1</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>27.8</td>
<td>11.5</td>
<td>28.0</td>
</tr>
<tr>
<td>MMP-3, ng/mL</td>
<td>418.5</td>
<td>132.6</td>
<td>365.2</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>105.3</td>
<td>92.2</td>
<td>101.9</td>
</tr>
</tbody>
</table>

### Measurements of arterial function

<table>
<thead>
<tr>
<th></th>
<th>GDM</th>
<th>Control</th>
<th>Overall p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1, mL/mmHg &gt; 100</td>
<td>14.4</td>
<td>3.5</td>
<td>15.5</td>
</tr>
<tr>
<td>C2, mL/mmHg &lt; 100</td>
<td>8.6</td>
<td>2.7</td>
<td>8.3</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>6.8</td>
<td>0.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Systolic cBP, mmHg</td>
<td>111.1</td>
<td>25.2</td>
<td>104.0</td>
</tr>
<tr>
<td>Diastolic cBP, mmHg</td>
<td>75.1</td>
<td>17.6</td>
<td>69.3</td>
</tr>
</tbody>
</table>

MetS affected participants in obese (BMI ≥ 30 kg/m^2) subgroups (GDM and non-GDM mothers combined) 4.4-fold more often than in non-obese (BMI < 30 kg/m^2) ones. BMI ≥ 30 kg/m^2 (OR 5.47, 95% CI 2.33–12.88; p < 0.001) was also significantly associated with an increased risk of MetS in univariate logistic regression analysis. Moreover, BMI ≥ 30 kg/m^2 was associated with a higher risk of MetS (OR 4.77, 95% CI 1.96–11.56; p = 0.001) in multiple linear regression analysis. The OR for previous GDM was 2.42 (95% CI 0.97–6.03; p = 0.059) in these analyses. These four subgroups did not differ significantly in family history of cardio- or cerebrovascular diseases, medical history, medication, contraception, physical activity or alcohol consumption. Obese subgroups showed significantly more pack-years of smoking than did the non-obese ones (Table 13). The subgroups did not differ significantly in perinatal outcomes either (data not shown). There was a major difference in lifetime weight loss (p < 0.001), with both obese GDM and obese control women having lost more weight than non-obese GDM and control women. There were significant differences in concentrations of fP-Gluc (p < 0.001) and fP-Insu (p < 0.001), and also in HOMA-IR index values (p < 0.001). The highest levels of fP-Insu were in the obese control group. These four subgroups did not differ as regards circulating oxLDL levels, but participants in obese groups did have higher serum concentrations of hsCRP than those in non-obese ones. Both systolic and diastolic cBP, as well as PWV, differed significantly in the four subgroups, but differences in both C1 and C2 values were nonsignificant.

5.5 Arterial stiffness in fertile women with metabolic syndrome (IV)

5.5.1 Women with metabolic syndrome and individually paired counterparts without the syndrome (IV)

From the original study population of 240 participants, there were 27 women with MetS in the follow-up study. Previously, nineteen of them had experienced GDM, while eight of them had not. In Study IV, twenty-seven women with MetS were compared with an individually matched counterpart without the syndrome. In addition to previous GDM, the counterparts without MetS were matched according to age, and serum concentrations of both LDL-C and TC.
Table 14. Matching parameters, background variables and laboratory findings in the follow-up study of women with MetS and their individually paired counterparts without the syndrome.

<table>
<thead>
<tr>
<th>Matching parameter</th>
<th>MetS n = 27</th>
<th>Paired counterparts n = 27</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>36.8 4.7</td>
<td>36.6 4.5</td>
<td>0.880</td>
</tr>
<tr>
<td>Previous GDM, n (%)</td>
<td>19 (70%)</td>
<td>19 (70%)</td>
<td>1.000</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.1 1.2</td>
<td>5.2 0.9</td>
<td>0.851</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.4 0.9</td>
<td>3.3 0.8</td>
<td>0.768</td>
</tr>
</tbody>
</table>

**Background variables**

| Current smokers   | 6 (22%)     | 4 (15%)                  | 0.076   |
| Pack-years of smoking | 4.1 8.7  | 1.9 4.8                  | 0.276   |
| Alcohol intake, g/day | 1.1 1.4  | 1.5 1.6                  | 0.242   |
| Weight loss during lifetime, kg | 30.4 31.4 | 28.0 35.2                | 0.657   |

**Follow-up study**

| BMI, kg/m²        | 33.5 6.2    | 28.9 5.0                  | 0.010   |
| Systolic BP, mmHg | 135.7 13.6  | 125.9 18.7                | 0.044   |
| Diastolic BP, mmHg| 78.4 8.1    | 73.0 12.1                 | 0.074   |
| Heart rate, beats per minute | 67.9 8.8  | 65.7 10.6                | 0.211   |

**Clinical chemistry and immunoassays**

| Leukocytes, 10⁹/L | 5.9 1.5   | 6.2 1.5                  | 0.536   |
| Hemoglobin, g/L   | 138.2 6.9 | 130.5 9.1                | 0.004   |
| Platelets, 10⁹/L  | 243.6 55.9| 267.3 65.8               | 0.194   |
| ALAT, U/L         | 32.3 24.1 | 22.2 20.5                | 0.022   |
| Creatinine, μmol/L| 65.3 9.0  | 64.6 5.4                 | 0.748   |
| Fibrinogen, g/L   | 3.4 0.9   | 3.7 1.1                  | 0.336   |
| U-AlbCre, mg/mmol | 0.7 0.4  | 0.5 0.3                  | 0.034   |
| fP-Insu, mU/L     | 9.0 5.9   | 6.4 4.3                  | 0.073   |
| HbA1c, mmol/mol   | 34.6 2.9  | 34.7 2.5                 | 1.000   |
| oxLDL, U/L        | 48.3 14.6 | 48.0 17.1                | 0.942   |
| hsCRP, mg/L       | 3.6 4.1   | 3.7 5.2                  | 0.516   |
| MMP-8, ng/mL      | 31.5 16.1 | 34.1 22.9                | 0.829   |
| MMP-9, ng/mL      | 414.2 137.1| 402.3 135.2              | 0.735   |
| TIMP-1, ng/mL     | 107.3 26.4| 95.7 30.3                | 0.102   |
| HOMA-IR           | 2.3 1.5   | 1.6 1.1                  | 0.046   |

ALAT: alanine transaminase; BMI: body mass index; fP-Insu: fasting plasma insulin; BP: blood pressure; GDM: gestational diabetes mellitus; HbA1c: glycosylated hemoglobin A1c; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; oxLDL: oxidized low-density lipoprotein; MetS: metabolic syndrome; MMP: matrix metalloproteinase; TC: total cholesterol; TIMP: tissue inhibitor of metalloproteinase; U-AlbCre: urinary albumin/creatinine ratio

In paired comparisons, there were no differences in family history of coronary heart disease, cerebrovascular disease or DM between the study groups (data not
shown). Neither were any differences found in medical history of diagnosed disorders or permanent medication for any chronic disease (data not shown). Further, there were no differences in current or pack-years of smoking, alcohol intake, heart rate or lifetime weight loss in individual pair-wise comparisons (Table 14). BMI was higher in MetS women, but their paired counterparts also had a high mean BMI. Background variables and laboratory findings in the follow-up study are illustrated in Table 14.

5.5.2 Arterial compliance, pulse wave velocity and central blood pressure (IV)

As measured by three different non-invasive methods, values of arterial function differed significantly between the fertile women with MetS and their individually paired counterparts without the syndrome (Table 15). Values of systemic arterial compliance, both C1 and C2, were lower among the MetS women. As measured by means of PWV, arterial stiffness was higher in the women with MetS than in their matched counterparts, as were both systolic and diastolic cBP (IV).

<table>
<thead>
<tr>
<th>Determinant of arterial stiffness</th>
<th>MetS n = 27</th>
<th>Paired counterparts n = 27</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1, mL/mmHg×10</td>
<td>15.1</td>
<td>16.1</td>
<td>0.034</td>
</tr>
<tr>
<td>C2, mL/mmHg×100</td>
<td>7.1</td>
<td>9.3</td>
<td>0.010</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>7.1</td>
<td>6.5</td>
<td>0.037</td>
</tr>
<tr>
<td>Systolic cBP, mmHg</td>
<td>120.9</td>
<td>111.5</td>
<td>0.031</td>
</tr>
<tr>
<td>Diastolic cBP, mmHg</td>
<td>81.3</td>
<td>74.1</td>
<td>0.035</td>
</tr>
</tbody>
</table>

cBP: central blood pressure; C1: large arterial compliance; C2: small arterial compliance; MetS: metabolic syndrome; PWV: pulse wave velocity
6 DISCUSSION

6.1 Long-term outcomes of mothers after gestational diabetes mellitus (I–III)

When studied on average 3.7 years after delivery, the women with a history of GDM had 2.4-fold increased prevalence of MetS and were more insulin resistant than those without. Serum concentrations of TIMP-1 were significantly upregulated after GDM, reflecting low-grade inflammation among this relatively young study population. Further, women with previous GDM had higher values of PWV, indicating that their arteries are less distensible than those in women with previous normoglycemic pregnancy.

6.1.1 Metabolic syndrome (I)

After a pregnancy affected by GDM, the prevalence of MetS was 2.4-fold higher than after a normoglycemic one. Additionally, multiple-adjusted analysis supported this finding. Recently, several other investigators have also observed a positive association between MetS and previous GDM (Akinci et al. 2011, Derbent et al. 2011, Di Cianni et al. 2007, Ijäs et al. 2013, Karoli et al. 2015, Lauenborg et al. 2005, Li et al. 2015, Mai et al. 2014, Noctor et al. 2015, Puhkala et al. 2013, Retnakaran et al. 2010, Tam et al. 2012, Verma et al. 2002, Wijeyaratne et al. 2006). In contrast, Tam et al. (2007) reported similar rates of MetS in Asian women with and without previous GDM (Tam et al. 2007). Using the NCEP ATP III criteria for MetS, in a trial conducted in Spain, Albareda et al. (2005) found a non-significant trend toward a higher prevalence of MetS after GDM (Albareda et al. 2005). The authors observed that the GDM group was biased toward normality, i.e., having a mean BMI of 23.1 kg/m², whereas control women volunteering in the study had an increased family history of DM. Furthermore, most of the GDM women were insulin-treated during the pregnancy, and some of them had glutamic acid decarboxylase autoantibodies, indicating latent autoimmune diabetes rather than insulin resistance (Albareda et al. 2005).
In 2014, in a systematic review, Xu et al. demonstrated that women with prior GDM have a 3.96-fold increased prevalence of MetS in the future versus those who have had a normal pregnancy. However, there are some factors that may modify the risk of developing MetS after GDM. For instance, ethnicity may affect susceptibility to MetS. After a diabetic pregnancy, Caucasian women demonstrated a higher incidence of developing MetS when compared with Asian ones, but only two out of fifteen studies were of Asian origin. There was also heterogeneity in the form of GDM treatment between the studies; one study out of fifteen involved only diet-treated GDM women, whereas the rates of drug-treated GDM women varied considerably in the other studies. Further, BMI modifies susceptibility to MetS. According to recent meta-analyses, the BMI-adjusted odds ratio for MetS after GDM is 2.53 (Y. Xu et al. 2014).

There was no such heterogeneity in the present study – all women were of Caucasian origin, and there was no statistically significant difference between the study cohorts in body weight or BMI. The current results support a relationship between previous GDM and MetS. These results are in agreement with most data reported earlier (Y. Xu et al. 2014).

6.1.2 Glucose metabolism and lipids (I & II)

Glucose regulation often normalizes after a pregnancy complicated by GDM. However, a sevenfold increased risk of T2DM after GDM is obvious (Bellamy et al. 2009, Kim et al. 2002). Guidelines suggest screening for T2DM between six to twelve weeks postpartum by using the 75-g, 2-h OGTT, and if the results are normal, it should then be repeated at least every three years (Gestational diabetes: Current Care Guidelines. 2013, Metzger et al. 2007). If measuring fP-Gluc alone, approximately 40% of individuals with diabetes are missed, and the test fails to identify those with IGT (Reinblatt et al. 2006). When screened between 6 weeks and 3 months postpartum, 13% to 32% of women with a recent history of GDM have IGT, which may later progress to T2DM (Ogonowski & Miazgowski. 2009, Retnakaran et al. 2009). In the current study, 39.0% of GDM women who underwent postpartum OGTT screening expressed glucose intolerance. Alarmingly, only 41 of the 120 women in the GDM cohort had a recommended OGTT after delivery. Although the importance of postpartum screening with an OGTT after recent GDM is known, screening rates are disappointingly low, varying globally between 14 and 61 percent (Clark et al. 2009, Shea et al. 2011).
Thus, there is evidence that as soon as in the early postpartum period following GDM impaired glucose tolerance is frequent, and women with a history of recent GDM have lower insulin sensitivity (Benhalima et al. 2014). In one study, when determined three months after delivery, women with prior GDM had higher blood glucose values and more unfavorable lipid profiles than women with a previous normoglycemic pregnancy, and the metabolic profile was worst in women requiring insulin (Kärkkäinen et al. 2013). When measured over three years after a pregnancy affected by GDM, there were significant differences in the values of fP-Gluc and HbA1c when compared with women with previous normoglycemic pregnancy. Further, women with previous GDM were still more insulin resistant than controls. The HOMA-IR index is a robust tool for the surrogate assessment of IR (Antuna-Puente et al. 2011, Lann & LeRoith. 2007), and it has also been proved to correlate with direct measurement of insulin sensitivity using the insulin clamp (Monzillo & Hamdy. 2003). Although the HOMA-IR method is mainly used to measure insulin sensitivity in large epidemiologic studies, a significant difference in HOMA-IR values was also found between the study groups in the present smaller study. The HOMA-IR results after GDM are in accordance with findings observed earlier (Saucedo et al. 2011).

When measuring lipids, only the concentrations of TGs differed significantly between GDM women and controls. There are no earlier studies on circulating oxLDL levels after a history of GDM. However, no connection was observed between previous GDM and circulating oxLDL in this setting of two cohorts. One explanation for this finding could be that during pregnancy the healthiest and leanest women do not attend OGTT screening in Finland. Thus, the control group lacked the women with the lowest GDM risk (Gestational diabetes. Current Care Guidelines. 2013). Another explanation might be that in the present study most of the GDM women had a mild form of insulin resistance with no medication during the pregnancy. According to the current data, from two to six years after delivery there is no correlation between a history of GDM and circulating levels of oxLDL.

6.1.3 Low-grade inflammation (III)

Levels of TIMP-1 were significantly upregulated after previous GDM, reflecting low-grade inflammation among this relatively healthy and young study population. No differences were found in circulating levels of MMP-8 or MMP-9 between the
two study cohorts. Further, there was no difference in levels of hsCRP either, when determined on average at 3.7 years after the index pregnancy.

Recent studies have revealed higher CRP and hsCRP levels in women with a history of GDM than in age-matched normal controls after a 1- or 5-year postpartum period (Heitritter et al. 2005, Lekva et al. 2016, Ozuguz et al. 2011). In contrast, Ajala et al. found no difference in circulating levels of CRP in women after previous GDM compared with controls 4–10 years postpartum (Ajala et al. 2015). Adipose tissue, especially visceral fat, is associated with increased low-grade inflammation (Wellen & Hotamisligil. 2003). In the current study, women with and without a history of GDM did not differ in BMI, which could partly explain the similar hsCRP levels between the study cohorts.

No earlier publications were found concerning female populations where levels of MMP-8, MMP-9 or TIMP-1 have been studied in connection with previous GDM. There is some evidence that glucose is capable of modulating the expression, production and activity of MMPs. For instance, endothelial cells cultured in hyperglycemic conditions present increased expression and activity of MMP-9 (Berg et al. 2011). One might postulate that during pregnancy GDM increases concentrations of MMPs and they in turn upregulate TIMP-1. After delivery, the concentrations of glucose, MMPs and TIMP-1 decrease consecutively. The prolonged upregulation of TIMP-1 found in this study without upregulated MMP levels may also be a result of the fact that upregulated TIMP-1 may suppress MMP-8 and MMP-9 levels. Further, a third explanation for prolonged TIMP-1 upregulation found in this work may be that prolonged elevation of TIMP-1 levels may mediate MMP-independent pro-inflammatory or growth-factor-like signaling functions contributing to low-grade inflammation (Hayakawa et al. 1992, Moore & Crocker. 2012, Stetler-Stevenson. 2008).

6.1.4 Arterial function (II & III)

When studied over three years after delivery, PWV was significantly higher among women with previous GDM, indicating that their arteries are less distensible than those in women with previous normoglycemic pregnancy. Previous GDM was also one of the significant determinants of PWV in multiple-adjusted analyses. These findings were supported by a (nonsignificant) difference in the large-artery compliance index, C1. On the other hand, neither compliance indices of small
arteries, C2, nor values of systolic or diastolic cBP differed between the study cohorts.

PWV is a measure of the speed at which a pulse wave travels through the arterial system and it has an inverse relationship with arterial distensibility (Nichols & O’Rourke. 2005). Carotid–femoral PWV gives a measure of regional stiffness, mostly in the aorta (Laurent et al. 2006). During an uncomplicated pregnancy, PWV may rise or remain unchanged (Edouard et al. 1998, Heitritter et al. 2005, Mersich et al. 2005, Oyama-Kato et al. 2006). Only a few trials have been carried out concerning a potential correlation between PWV and a history of GDM. In one small study (n = 30), at an average of eight weeks after delivery, there were no differences in values of upper-limb PWV between women with and without previous GDM (Davenport et al. 2012). At 5-year follow-up, in a study by Lekva et al. (2015) (n = 284) an enhanced CVD risk was reported as reflected in elevated aortic PWV after previous GDM diagnosed using the old criteria of the World Health Organization established in 1999. However, such a correlation with PWV values when using IADPSG diagnostic criteria was not observed (Lekva et al. 2015, WHO. 2014). Using diagnostic criteria of GDM similar to those of the IADPSG (Gestational diabetes. Current Care Guidelines. 2013), a significant increase in PWV in women with previous GDM was revealed in the current study. Further, this finding was supported by the results of multiple linear regression analysis. The results are in accordance with those of Tam et al., who reported higher PWV in women with a history of GDM (n = 608) followed up at a median of six years postpartum (Tam, Ma, Chan et al. 2012). In contrast to these findings, Heitritter et al. detected no difference in PWV in women (n = 48) at an average of one year after previous GDM compared with women who had had normoglycemic pregnancies (Heitritter et al. 2005).

When measuring vascular function three months postpartum using the ambulatory arterial stiffness index in women with and without previous GDM, Kärkkäinen et al. (2013) observed a tendency towards increased arterial stiffness in women requiring insulin during the index pregnancy (Kärkkäinen et al. 2013). Further, using devices to measure macro- and microvascular function different to those used in current studies, Hu et al. (1998) noticed evidence of increased wall stiffness in the common carotid artery two to four years after a pregnancy complicated by GDM (J. Hu et al. 1998).

In Studies II & III, there were no significant differences in C1 or C2 values nor systolic or diastolic cBP values between the GDM cases and controls. In contrast to PWV as a measure of regional stiffness, arterial compliance (both C1 and C2)
reflects systemic stiffness, taking into account inertia of the blood, proximal and distal pressure, and also systemic vascular resistance (Cohn et al. 1995). In an earlier study, no difference was found in vascular function measured by using an HDI/PulseWave\textsuperscript{TM}CR-2000 system in women with a history of GDM when compared with healthy controls 4–10 years postpartum (Ajala et al. 2015). Tam et al. (2012a) reported no significant difference in the rate of hypertension, but systolic cBP (106 ± 12 mmHg vs. 102 ± 13 mmHg; p = 0.03), assessed by using a SphygmoCor\textsuperscript{®} device, was increased in women with history of GDM. Their cBP findings suggested a major risk of subclinical atherosclerosis among women with a history of GDM despite the fact that brachial BP appeared to be normal at the time of follow-up, at a median of six years postpartum (Tam et al. 2012).

Seemingly, GDM is not associated with indices of arterial compliance (C1 and C2) nor cBP values 2–6 years after delivery in a setting of two cohorts with similar body weight and BMI. However, when (gold standard) PWV was used, previous GDM was associated with stiffer arteries.

### 6.2 Effect of obesity (I–III)

The epidemic of overweight conditions and obesity, in other words an overload of adipose tissue, has caused a dramatic growth in the number of individuals with several comorbidities including metabolic and premature CV disease (Kivimäki et al. 2017, Obesity (adult). Current Care Guidelines. 2013, van Greevenbroek et al. 2013). Obesity, particularly central obesity leading to accumulation of intra-abdominal adipose tissue is strongly related to metabolic disease. The results of several trials have linked IR with the accumulation of visceral fat (Wagenknecht et al. 2003). Impaired insulin signaling leads to an increased demand for insulin and consequently, increased insulin production by the pancreatic $\beta$-cells, a process known as compensatory $\beta$-cell function. At first, obesity-induced IR leads to increased levels of insulin, but if the condition is prolonged or worsens, $\beta$-cells may become fatigued and no longer able to meet the high demand of producing insulin. Eventually, hepatic and peripheral glucose disposal will become insufficient and gluconeogenesis in the liver increases, leading subsequently to higher concentrations of glucose, and finally, to the development of T2DM (van Greevenbroek et al. 2013). Further, accumulation of intra-abdominal fat is related to a low-grade inflammatory response, which may lead to vascular dysfunction (Takeoka et al. 2016, van Greevenbroek et al. 2013).
When we divided the whole study population of 240 women into four subgroups according to BMI and a previous diagnosis of GDM, most of the study outcomes were more evident in obese women than in non-obese ones. Further, the influence of obesity frequently exceeded that of previous GDM. However, GDM seemed to have an additive influence on CVD risk factors among obese women. When studying the effect of obesity independent of the cut-off value of BMI, MetS affected women in obese (BMI ≥ 30 kg/m$^2$) subgroups 4.4-fold more often than in non-obese (BMI < 30 kg/m$^2$) ones independent of previous GDM history, indicating metabolic abnormalities in obese groups. Further, a major difference was found in lifetime weight loss, both obese GDM and obese control women having lost more weight than non-obese GDM and control women. So called “yo-yo” dieting or weight cycling, meaning initially successful weight loss followed by weight regain is correlated with excess body weight, and, particularly, abdominal fat accumulation (Cereda et al. 2011).

A variety of CVD risk factors such as increased levels of LDL-C and TGs, as well as decreased concentrations of HDL-C were more obvious in women with high BMI. In contrast, the four subgroups did not differ significantly as regards circulating levels of oLDL. However, differences in concentrations of fP-Gluc and fP-Insu, and also in HOMA-IR index values were significant between the cohorts. Multiple-adjusted analyses highlighted the association between BMI and HOMA-IR values. One could postulate that fP-Insu levels were higher in the obese GDM group than in the obese control group. In subgroup analyses, however, the obese control group seemed to have the highest concentrations of fP-Insu and the highest HOMA-IR index values, although their circulating concentrations of fP-Gluc were significantly lower than in both of the GDM groups. GDM places affected women at a sevenfold risk of developing T2DM (Bellamy et al. 2009); thus some of the women with a history of GDM may have developed prolonged IR with β-cell dysfunction, leading to decreased concentrations of fP-Insu. Further, women with previous GDM may already have a prediabetic condition or even undiagnosed T2DM. Moreover, according to the current results, the obese control women with increased levels of fP-Insu had compensatory β-cell function, which, however, in the long term does not prevent the future development of T2DM. Genetic variation may influence gene expression by way of different mechanisms (Parikh et al. 2009), which may partly explain the results in the obese control cohort. For instance, a Pro12Ala polymorphism has been associated with increased insulin sensitivity and thereby provides protection against T2DM (Deeb et al. 1998).
In Study III, the women in the obese subgroups had higher serum levels of hsCRP than those in the non-obese ones, reflecting a low-grade inflammatory state among obese women. However, differences in concentrations of MMP-8, MMP-9 and TIMP-1 did not reach statistical significance. Both systolic and diastolic cBP, as well as PWV, differed significantly in the four subgroups, indicating less distensible vessels in obese groups. In conclusion, these results highlight the fact that obesity may lead to a low-grade inflammatory state, and, further, vascular dysfunction (Takeoka et al. 2016, van Greevenbroek et al. 2013).

Once a person becomes obese, it is challenging to decrease body weight (Ogden et al. 2014). This emphasizes the necessity of counseling a healthy lifestyle among women, not only those with previous GDM, but also with obesity, in order to prevent complications of premature CV diseases and to reduce the probability of developing T2DM later in life. In fact, treatment of obesity should already be of concern before childbearing age, since overweight conditions and obesity in childhood are usually maintained in adulthood (A. S. Singh et al. 2008).

6.3 Arterial stiffness in fertile women with MetS (IV)

The validity of a diagnosis of MetS has occasionally been the subject of severe criticism (Balkau et al. 2002, Bauduceau et al. 2007, Borch-Johnsen & Wareham. 2010, Kahn et al. 2005, Mente et al. 2010, Simmons et al. 2010, Woodward & Tunstall-Pedoe. 2009). The crucial concerns are the debatable pathophysiology of the syndrome, the use of discontinuous thresholds to determine abnormalities, the presence of different definitions, the exclusion of other important CVD risk factors such as age, family history or LDL-C, and, further, the absence of any particular treatment for the syndrome, except weight loss (Borch-Johnsen & Wareham. 2010, Simmons et al. 2010). Moreover, although there is more knowledge regarding pathophysiological differences between genders in the prevalence of MetS components, women are underrepresented in clinical trials, which may negatively affect the interpretation of epidemiological and clinical evidence (Santilli et al. 2017). In the present cross-sectional Study IV concerning individually paired women with and without MetS, there were increased PWV values among women with MetS when compared with women without the syndrome. This finding suggests that MetS in fertile-aged women is associated with increased arterial stiffness. Further, women with MetS had increased cBP, as well as decreased C1 and C2 values when compared with their counterparts without the
syndrome, thus providing further support for the presence of arterial stiffness among women with MetS.

As mentioned earlier, increased PWV – as a measure of arterial stiffening – is a powerful predictor of CVD events and mortality (Vlachopoulos et al. 2010). There are several potential explanations for the finding of higher PWV in women with MetS. Small dense LDL (sdLDL) particles, reflecting poor-quality LDL, known to be associated with MetS, and hypertriglyceridemia, have been found to be important predictors of atherosclerosis (Y. Cho et al. 2015, Hoogeveen et al. 2014). Like sdLDL particles, circulating triglyceride-rich lipoproteins may also induce endothelial dysfunction (Lucero et al. 2016, Wakatsuki et al. 2004). Chronic hyperglycemia and hyperinsulinemia promote the development of arterial-wall hypertrophy by increasing local activity of the renin-angiotensin-aldosterone system (Zieman et al. 2005). Furthermore, high BP stimulates excessive collagen production in the arterial wall and IR promotes the formation of advanced glycation end-products and collagen cross-linking (Prenner & Chirinos. 2015, Zieman et al. 2005). In MetS the vasodilatory property of insulin is impaired. Further, the increased concentration of free fatty acids can also lead to endothelial dysfunction (Zieman et al. 2005). MetS can also be considered to be a pro-inflammatory state, which could cause endothelial dysfunction (Tzio malos et al. 2010). All these changes in arterial-wall function and structure, and, further, perivascular fat, have an unfavorable impact on the softening capabilities of arteries, thus increasing arterial stiffness (Lim & Meigs. 2013, Tzio malos et al. 2010, Zieman et al. 2005).

Carotid–femoral PWV is considered to be a gold standard in the evaluation of arterial dysfunction (Cheung. 2010, Hodes et al. 1995, Laurent et al. 2006). Arterial stiffness can also be determined by measuring cBP or compliance of large (C1) and small (C2) arteries (Cohn et al. 1995, Hodes et al. 1995). As discussed in a consensus document by Agabiti-Rosei et al. (2007), increased cBP has been shown to be related to CVD risk in apparently healthy subjects and in patients with atherosclerotic disease (Agabiti-Rosei et al. 2007). Moreover, decreased values of C1 and C2 have been found to be correlated with MetS (Ge et al. 2008) and increased CVD risk as approximated by using SCORE and FINRISK risk models (Pohjantähti-Maaroos et al. 2012). In the present study fertile-aged women with MetS had higher cBP, and lower C1 and C2 values when compared with women without the syndrome. This provides further evidence of the negative effects of MetS on arterial stiffness among fertile-aged women. Between the study groups there was a small but significant difference in microalbuminuria. As a marker of
endothelial dysfunction (Monhart. 2011), this finding also highlights the effect of MetS on arterial stiffness.

In several previous studies, MetS has been shown to be related to an elevated risk of CVD (G. Reaven. 1988, G. M. Reaven. 1992, Trevisan et al. 1998, Y. Xu et al. 2014), and, further, the risk of CVD associated with MetS is clearly greater than the risk associated with any of its individual elements (Isomaa et al. 2001). Moreover, it has been suggested that MetS could be a valuable public-health tool, as it can be used to identify high-risk individuals at a young age (Cameron et al. 2009). The current results, showing increased arterial stiffness in fertile-aged women with MetS as measured by three different methods, even when their counterparts are matched according to many other well-known CVD risk factors, strongly support the clinical use of MetS as a tool for CVD risk assessment, particularly among fertile-aged women.

6.4 Strengths and limitations of the study

The study population in observational Studies I to III – two cohorts of women with and without previous GDM – was well characterized, with a similar age range and time from delivery to the follow-up study. Moreover, there was no significant difference in BMI between the study groups, and all women in both the GDM and the control group had attended OGTT screening during the previous (index) pregnancy. In Finland, OGTT screening for GDM is offered to all gravidas, except those who are at low risk (Gestational diabetes. Current Care Guidelines. 2013). As 2.7–20% of women diagnosed with GDM have no risk factors for it (Avalos et al. 2013, Chevalier et al. 2011), the exclusion of low-risk women without OGTT screening during the index pregnancy confirms that there was no hidden glucose intolerance in the control group. Finnish national diagnostic criteria for GDM are similar to those used internationally (WHO. 2014), and the NCEP ATP III diagnostic criteria for MetS are practical, and widely used for clinical diagnosis and management (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002). Additionally, the NCEP ATP III definition of MetS confers a significantly higher risk of vascular events than the IDF definition (International Diabetes Federation. 2006). Further, in (cross-sectional) Study IV, the paired fertile-aged women with and without MetS had been strictly selected with identical traditional risk factors of CVD. Although the number of participants
in Study IV was relatively small, the number of women was large enough to show meaningful and statistically significant differences between the matched groups.

Strengths of the current study include validated determinations of laboratory analytes and standardized measurements of arterial stiffness. For example, determination of systemic arterial compliance by using HDI/PulseWave™CR-2000 equipment is widely carried out, and, in particular, carotid–femoral PWV is accepted as a gold standard measurement of arterial stiffness. Further, PWV has the greatest amount of epidemiological evidence of its predictive value as regards CVD events, and the methodology does not require special technical expertise (Laurent et al. 2006). In clinical chemistry, a highly sensitive immunonephelometric method was used for assay of hsCRP (Chenillot et al. 2000, Sanchez et al. 2002). Moreover, levels of oxLDL were defined as originally described by Holvoet et al. (Holvoet et al. 1998, Holvoet et al. 2001). The concentrations of MMP-8, MMP-9 and TIMP-1 were measured by specific immunoassays earlier found to be eligible for diagnosis and follow-up of systemic low-grade inflammation (Lauhio et al. 1994, Lauhio et al. 1995, Lauhio et al. 2011, Lauhio et al. 2011, Lauhio et al. 2016, Pussinen et al. 2013, Rautelin et al. 2009, Sorsa et al. 2006, Sorsa et al. 2011, Tuomainen et al. 2007).

In Finland, low-risk parturients do not attend OGTT screening during pregnancy (Gestational diabetes. Current Care Guidelines. 2013). Hence, during the study period, 42.5% of gravidas had undergone OGTT screening for GDM in Kanta-Häme Central Hospital, meaning that 57.5% of pregnant women at that time were at the lowest risk and thus excluded from this study. As the healthiest and leanest women were excluded from the study, some of the nonsignificant findings may be compromised (I–III). Having only a few years from delivery to the follow-up study allowed us to observe possible early CV changes between the study cohorts, but it may be one of study limitations, since major differences between the cohorts are probably more easily observable later in life. Although the estimation of insulin sensitivity using HOMA-IR is less precise than insulin clamp measurement – the gold standard for analyzing IR – HOMA-IR can give a good measure of IR in non-diabetic individuals (Monzillo & Hamdy. 2003).

In Study I, an ambiguous matter was the BMI cut-off point of 27 kg/m², because obesity is often classified as BMI of ≥ 30 kg/m² (Report of a WHO consultation. 2000). Medicines agencies both in Europe and in the USA define a cut-off point of BMI of 27 kg/m² when investigating medication for obesity, as discussed in detail earlier (Colman. 2012). Further, according to the FINRISK 2012 Study, mean BMI among women aged 25–74 years is 26.8 kg/m² in Finland.
(Borodulin et al. 2014). In subgroup analysis of Study I, BMI was used to divide the study population into two halves, intending to reveal the effect of excess body weight or preobesity on CVD risk factors. The cut-off point of BMI we used in Study I fairly well represents average BMI among Finnish women. However, because of the equivocal nature of the BMI cut-off level in Study I, we decided to use a more commonly accepted classification of obesity in Studies II and III. Furthermore, new subgroup analyses of the material in Study I were carried out with a BMI cut-off point of 30 kg/m², as reported in the Results section.

6.5 Future considerations

In women, CVD is the leading cause of death globally (S. K. Lee et al. 2017). Further, based on unequivocal evidence, a history of GDM should be seen as a powerful CVD risk factor unique to women (Mosca et al. 2011). Hereafter, investigations should be focused on recognizing and comprehending the mechanisms that lead to future CVD in these women; resolving whether pregnancy uncovers a prevalent predisposition to CV disease or increases the risk of future CVD (Lind et al. 2014).

After delivery, women with a history of GDM, both obese and nonobese ones, have greater arterial stiffness and decreased endothelial function. However, the actual mechanisms contributing to a risk of vascular dysfunction remain uncertain. Further studies with greater numbers of participants are needed to identify and validate biomarkers of CVD risk before development of T2DM, MetS or CVD. Investigating the effect of the duration of a variety of CVD risk factors after an index pregnancy with GDM may have implications for postpartum screening. Longitudinal trials may help to determine correlations among glycemic levels, IR, endothelial arterial function and CVD (Jensen et al. 2016).

Considerable work is also needed to reveal genetic mechanisms underlying previous GDM and its evolution to T2DM after pregnancy. Genetic predisposition and metabolic dysfunction are two common factors behind T2DM. Recent estimations of T2DM heritability have varied from 25% to 80% (Prasad & Groop. 2015). In the future, genetic research may help us to identify women whose β-cells respond poorly to IR, as well as women who develop weak insulin secretion for reasons unrelated to IR. Studies of gene–environmental interactions, i.e. epigenetics, and further investigations of insulin action in fat and muscle may
identify causes of IR, particularly in relation to an overload of adipose tissue (Buchanan & Xiang, 2005).

Although the importance of postpartum OGTT screening after GDM is well known, the rate of attendance at follow-up tests remains disappointing low in routine clinical practice (Clark et al. 2009, Shea et al. 2011). Therefore, further research is required to identify elements that have an impact on the health beliefs and behaviors of women with previous GDM (Jones et al. 2009). There is a necessity for interventions to enhance awareness of the personal risk of future development of T2DM in GDM women. Public campaigns might help to improve risk-awareness of GDM women. Additionally, pharmacological trials are still needed to approximate the cost-effectiveness of the prevention of both CVD and T2DM (Di Cianni et al. 2010). For example, metformin, which was originally used for the treatment of T2DM, has now also been proven to prevent or delay diabetes. This may serve as an important tool in battling the growing epidemic of diabetes (Aroda et al. 2017). Long-standing, continuous programs addressed to women previously affected by GDM could be performed in order to encourage them to regularly check glucose and lipid metabolism, BP and other parameters aimed at improving their health (Di Cianni et al. 2010). Such attention could potentially offset the significant morbidity associated with chronic diabetes (Kim. 2010b).

Unfortunately, the low rate of participation in postpartum follow-up among GDM women also suggests decreased risk-awareness among physicians: healthcare providers may not recognize GDM as the first warning sign of predisposition to T2DM, MetS and CVD. With identical risk profiles, intermediate-risk women, in comparison with men, have been found to be more likely to be assessed as lower-risk individuals by primary-care physicians, obstetricians or gynecologists, and cardiologists (Mosca et al. 2005). Therefore, a focus on education, not only of patients, but also of physicians as regards primary prevention in women is necessary. Furthermore, clinical research particularly focused on women is needed, as the majority of CVD trials have been carried out among men. Updated guidelines on prevention of CV diseases in women would help to assist as regards appropriate clinical and laboratory determinations and to optimize atherosclerotic CVD prevention in half of the world’s population (S. K. Lee et al. 2017).
The purpose of this work was to study arterial stiffness and non-traditional biomarkers of CVD risk in order to explain the higher CVD risk in women with previous GDM. Another aim was to observe the effect of obesity on the results. Moreover, a target was to reveal the utility of MetS determination when estimating individual CVD risk. Therefore, differences in arterial stiffness and CVD risk components were explored in individually paired fertile women with and without MetS.

The main findings and conclusions were:

1. The prevalence of MetS was 2.4-fold higher after GDM than after normal pregnancy. Previous GDM was also associated with an increased risk of MetS in univariate logistic regression analysis. Further, multiple-adjusted analysis supported this main finding (I).

2. OxLDL concentrations and cBP did not differ between women with and without previous GDM, but HOMA-IR values were significantly higher in women with previous GDM than in controls. In a more than three-year period after delivery women with GDM were more insulin resistant than controls (II).

3. No differences were found in the serum concentrations of MMP-8, MMP-9 or hsCRP between women with and without previous GDM. On the other hand, serum levels of TIMP-1 were significantly upregulated after previous GDM, reflecting low-grade inflammation among the GDM population. Compliance indices (C1 and C2) did not differ between the GDM women and controls. However, after pregnancy complicated by GDM, PWV was significantly higher than after normal pregnancy, indicating that the arteries in women with previous GDM are less distensible than those in women with previous normoglycemic pregnancy. Further, this last finding was supported in multiple-adjusted analyses (III).
4. CVD risk factors such as increased levels of LDL-C and TGs as well as decreased HDL-C concentrations were more common in obese women than in non-obese subgroups. Additionally, HOMA-IR values, concentrations of hsCRP, systolic and diastolic cBP, and values of PWV were significantly higher in obese subgroups compared with non-obese ones. As regards risk factors of CVD, the influence of obesity frequently exceeded that of GDM. However, previous GDM seemed to have an additive influence on CVD risk factors among both obese and non-obese women (I–III).

5. As measured by three non-invasive methods, fertile women with MetS had increased arterial stiffness when compared with individually paired women without the syndrome. The results support the clinical use of MetS when revealing increased individual CVD risks, particularly among fertile-aged women (IV).

7.1 Challenge of long-term follow-up after gestational diabetes mellitus

T2DM is both a personal and public health disaster if not diagnosed in time, treated without delay and managed appropriately. Over 60% of instances of mortality and disability, including leg amputation, heart and kidney diseases, stroke, cancer as well as depression are causally related to diabetes (Chan et al. 2009, Ramachandran et al. 2010, Rao Kondapally Seshasai et al. 2011). According to the Finnish National Institute for Health and Welfare, the annual cost of T2DM treatment without any comorbidities is 1300 € per person, and with comorbidities, 5700 € per person (Finnish National Institute for Health and Welfare. 2016). Even before development of T2DM, women with prior GDM have significant differences in CVD risk factors when compared with those who do not have such a history. Postpartum screening for glucose intolerance and efforts to minimize modifiable CVD risk factors, including central obesity, dyslipidemia, and elevated BP should be the most effective measures for lowering the risks of both T2DM and CVD in women (Karoli et al. 2015). However, as only about 34% of women with a history of GDM participate in the suggested OGTT screening postpartum, potentially two-thirds of T2DM diagnoses are not going to be made in time. One
way to improve attendance at postpartum screening might be to combine followup studies of the mothers with appointments at child health centers.

Besides T2DM, there is a global epidemic of obesity. Around half of all women of reproductive age are either overweight or obese. Excessive gestational weight gain and postpartum weight retention may play a significant role in long-term obesity. Maternal obesity increases the risk of pregnancy-related complications such as preeclampsia, GDM and the rate of cesarean section. Childhood obesity is a further long-term complication of maternal obesity for offspring, which may persist into adulthood (Spencer et al. 2015). The relationship between GDM and hypertension or CV disease is evident. Further, overweight conditions and obesity seem to have an even stronger association with CVD risk. These facts, as well as the results of the current work, suggest a need for effective interventions to manage both these conditions in order to improve the health of women, not only those with a history of GDM, but also those who are overweight or obese (Kaul et al. 2015).

In Finland, there is no consensus of opinion regarding how to monitor obese women after normal pregnancy. According to the current results, one should consider screening unaffected obese women for CVD risk factors and impaired glucose tolerance after delivery. Paying attention to individuals with pathological OGTT results as well as an overweight condition during and after pregnancy helps healthcare providers to recognize women who may be at risk of developing MetS, T2DM or CVD later in life. This emphasizes the necessity of counseling a healthy lifestyle among women with obesity or previous GDM in order to prevent premature complications of CV diseases and decrease the burden of developing T2DM in the future.

After several decades of research, there is still no unified global approach to GDM (Negrato & Gomes. 2013). If the rate of attendance at lifetime follow-up among GDM women could be improved, there should also be time to come to an agreement on a global guideline on universal screening for GDM. Pregnancy offers a unique window through which women at risk of future T2DM, MetS or CVD may be identified. Healthcare professionals including general practitioners, obstetricians and gynecologists should not miss this opportunity to implement health monitoring, lifestyle modifications, and other forms of intervention that will help reduce the burden of CVD and metabolic morbidity (Lind et al. 2014). Both long- and short-term improvement of postpartum follow-up is crucial to battle against the growing epidemic of diabetes and obesity.
This research was conducted at the Department of Obstetrics and Gynecology, Kanta-Häme Central Hospital, and Linnan Klinikka, Hämeenlinna. The work was supported financially by grants from the Finnish Cultural Foundation, Häme Regional Fund, the Faculty of Medicine and Life Sciences at the University of Tampere, and the Ministry of Health and Social Welfare in Finland via Medical Research Funds of both Kanta-Häme Central Hospital and Tampere University Hospital. I want to acknowledge both the study places and the financial supporters of this work.

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At the end of May 2018 in Hämeenlinna,

with love and hugs,

Tiina Vilmi-Kerälä
9 REFERENCES


Booth GL, Kapral MK, Fung K & Tu JV. (2006) Relation between age and cardiovascular disease in men and women with diabetes compared with non-


10 ORIGINAL PUBLICATIONS
The risk of metabolic syndrome after gestational diabetes mellitus – a hospital-based cohort study

Tiina Vilmi-Kerälä1,2,3*, Outi Palomäki2, Merja Vainio3, Jukka Uotila1,2 and Ari Palomäki1,4

Abstract

Background: Women with gestational diabetes mellitus (GDM) are at an increased risk of developing metabolic syndrome (MetS) after delivery. Recently, the prevalence of both GDM and MetS has increased worldwide, in parallel with obesity. We investigated whether the presentation of MetS and its clinical features among women with previous GDM differs from that among those with normal glucose tolerance during pregnancy, and whether excess body weight affects the results.

Methods: This hospital-based study of two cohorts was performed in Kanta-Häme Central Hospital, Finland. 120 women with a history of GDM and 120 women with a history of normal glucose metabolism during pregnancy, all aged between 25 and 46 were enrolled. They all underwent physical examination and had baseline blood samples taken. All 240 women were also included in subgroup analyses to study the effect of excess body weight on the results.

Results: Although the groups did not differ in body mass index (BMI; p = 0.069), the risk of developing MetS after pregnancy complicated by GDM was significantly higher than after normal pregnancy, 19 vs. 8 cases (p = 0.039). Fasting glucose (p < 0.001) and triglyceride levels (p < 0.001) were significantly higher in women affected. In subgroup analysis, cardiovascular risk factors were more common in participants with high BMI than in those with previous gestational diabetes.

Conclusions: The risk of MetS was 2.4-fold higher after GDM than after normal pregnancy. Cardiovascular risk factors were more common in participants with high BMI than in those with previous GDM. Multivariate analysis supported the main findings. Weight control is important in preventing MetS after delivery.

Keywords: Gestational diabetes mellitus, Metabolic syndrome, Body mass index, Body weight excess, Cardiovascular risk factors

Introduction

The prevalence of gestational diabetes mellitus (GDM) has increased globally in recent decades along with increasing rates of obesity and inactive lifestyles [1,2]. In Finland, GDM affected 15.0% of pregnancies in 2013 [1]. Glucose intolerance normalizes after delivery in most cases [3,4], but women with a history of GDM have at least a sevenfold risk of developing type 2 diabetes in the future [5]. Affected women are also at an increased risk of developing cardiovascular disease or metabolic syndrome (MetS) years after the pregnancy [6-9].

Metabolic syndrome is an international health problem considered to be the result of concomitant accumulation of abdominal obesity, hypertension, dyslipidaemia and abnormal glucose tolerance or diabetes [10]. In recent decades, the prevalence of MetS has rapidly increased in parallel with sedentary lifestyles [6], leading to major healthcare costs. The chance of developing cardiovascular disease is six to eight times higher and that of mortality related to cardiovascular disease two to three times higher among the MetS population than among healthy controls [11-14].

Gestational diabetes mellitus shares common features with MetS, including dyslipidaemia, insulin resistance and endothelial dysfunction [15-19]. Several studies have revealed an increased risk of MetS in association with a history of GDM [7,20,21]. For example, a Danish study...
demonstrated that the prevalence of MetS in women with a history of GDM was threefold higher than in the general age-matched population [7]. However, other studies have shown contrasting results, with no association between GDM and MetS [22,23].

Women’s health after GDM has been widely studied. However, the effect of an overweight condition on health after GDM or after normal pregnancy is less well known. The aim of our hospital-based study of two age-matched cohorts was to reveal whether or not the presentation of MetS and its individual variables among women with previous GDM differs from those with normal glucose metabolism a few years after delivery. In this first study of the Hämeenlinna GDM Research Programme, we also wanted to investigate if there is a difference in clinical features between the groups and whether excess body weight affects the results.

**Methods**

We investigated a total of 120 parturients from our area aged 25 to 46 years and with a history of GDM during the index pregnancy and we compared them with 120 age-matched women with normal glucose metabolism during pregnancy. Power analyses were conducted to estimate the required number of participants. Concerning continuous variables, we worked on a difference of 10% with a standard deviation of 25% (Cohen’s d = 0.40). Regarding the presentation of MetS the expected proportions were 10% and 25%. When the significance level was set at 5% and power at 80%, the estimated numbers of participants as regards continuous and categorical variables were 99 and 100 in both groups, respectively. In Kanta-Häme Central Hospital, Finland, there are approximately 1700 deliveries annually. The electronic database of the hospital was used to pick up the cases and controls. Both recruitment and examinations were carried out between August 2011 and July 2014.

**Table 1 Characteristics of the index pregnancy in the GDM and control groups**

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<tr>
<td>- 0 h, mmol/L</td>
<td>5.4 ± 0.5</td>
<td>4.7 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>- 1 h, mmol/L</td>
<td>9.5 ± 2.3</td>
<td>7.1 ± 1.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>- 2 h, mmol/L</td>
<td>7.7 ± 2.0</td>
<td>5.8 ± 1.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Pregnancy disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Gestational hypertension, n (%)</td>
<td>12 (10%)</td>
<td>6 (5%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Pre-eclampsia, n (%)</td>
<td>7 (5.8%)</td>
<td>2 (1.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Glucosuria, n (%)</td>
<td>25 (20.8%)</td>
<td>4 (3.3%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>- Proteinuria, n (%)</td>
<td>19 (15.8%)</td>
<td>7 (5.8%)</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>Induction of delivery, n (%)</strong></td>
<td>42 (35.0%)</td>
<td>26 (21.7%)</td>
<td>0.031</td>
</tr>
<tr>
<td><strong>Caesarean section, n (%)</strong></td>
<td>29 (24.2%)</td>
<td>21 (17.5%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Perinatal outcome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Gestational age, days</td>
<td>277.1 ± 9.5</td>
<td>278.8 ± 10.4</td>
<td>NS</td>
</tr>
<tr>
<td>- Birth weight of the child, g</td>
<td>3633 ± 519</td>
<td>3540 ± 471</td>
<td>NS</td>
</tr>
<tr>
<td>- Apgar score at one minute</td>
<td>8.6 ± 1.2</td>
<td>8.7 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>- Apgar score at five minute</td>
<td>9.3 ± 0.8</td>
<td>9.3 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>- Umbilical blood arterial pH</td>
<td>7.29 ± 0.1</td>
<td>7.28 ± 0.1</td>
<td>NS (0.054)</td>
</tr>
<tr>
<td>- Umbilical blood venous pH</td>
<td>7.35 ± 0.1</td>
<td>7.35 ± 0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2 Clinical characteristics in the GDM and control groups

<table>
<thead>
<tr>
<th></th>
<th>GDM (n = 120)</th>
<th>Control (n = 120)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at follow-up, years</strong></td>
<td>35.8 ± 4.4</td>
<td>35.9 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Family history of</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Coronary heart disease, n (%)</td>
<td>20 (16.7%)</td>
<td>23 (19.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Cerebrovascular disease, n (%)</td>
<td>15 (12.5%)</td>
<td>5 (4.2%)</td>
<td>0.033</td>
</tr>
<tr>
<td>- Diabetes mellitus, n (%)</td>
<td>32 (26.7%)</td>
<td>27 (22.5%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Diagnosed disorder, n (%)</strong></td>
<td>52 (43.3%)</td>
<td>45 (37.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Hypertension, n (%)</td>
<td>3 (2.5%)</td>
<td>5 (4.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Type 1 diabetes mellitus, n (%)</td>
<td>2 (1.7%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Type 2 diabetes mellitus, n (%)</td>
<td>1 (0.8%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Polycystic ovary syndrome, n (%)</td>
<td>8 (6.7%)</td>
<td>1 (0.8%)</td>
<td>0.036</td>
</tr>
<tr>
<td><strong>Permanent medication for any chronic disease, n (%)</strong></td>
<td>43 (35.8%)</td>
<td>35 (29.2%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Current, n (%)</td>
<td>24 (20.0%)</td>
<td>12 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Former, n (%)</td>
<td>45 (37.5%)</td>
<td>37 (30.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Never, n (%)</td>
<td>51 (42.5%)</td>
<td>71 (59.2%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BMI, kg/m2</strong></td>
<td>28.3 ± 5.0</td>
<td>27.5 ± 5.4</td>
<td>NS (0.069)</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>96.8 ± 13.0</td>
<td>92.5 ± 12.6</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Systolic blood pressure, mmHg</strong></td>
<td>122.4 ± 12.5</td>
<td>119.0 ± 11.5</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure, mmHg</strong></td>
<td>73.5 ± 9.0</td>
<td>71.8 ± 8.7</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Heart rate, beats per minute</strong></td>
<td>65.9 ± 9.1</td>
<td>63.8 ± 9.6</td>
<td>0.017</td>
</tr>
<tr>
<td><strong>MetS, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Waist circumference &gt; 88 cm, n (%)</td>
<td>19 (15.8%)</td>
<td>8 (6.7%)</td>
<td>0.039</td>
</tr>
<tr>
<td>- Blood pressure ≥ 130/85 mmHg, n (%)</td>
<td>89 (74.2%)</td>
<td>73 (60.8%)</td>
<td>0.038</td>
</tr>
<tr>
<td>- HDL cholesterol &lt; 1.30 mmol/L, n (%)</td>
<td>35 (29.2%)</td>
<td>25 (20.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>- Triglycerides ≥ 1.7 mmol/L, n (%)</td>
<td>12 (10.0%)</td>
<td>5 (4.2%)</td>
<td>NS (0.084)</td>
</tr>
<tr>
<td>- Glucose ≥ 6.1 mmol/L or diabetes, n (%)</td>
<td>18 (15.0%)</td>
<td>4 (3.3%)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD if not mentioned otherwise. OGTT: Oral glucose tolerance test. Metabolic syndrome and separate variables defined by NCEP.
Inclusion criteria were as follows:

– Index pregnancy and delivery 2–6 years before participating in the study
– GDM group: GDM defined as a pathological value in the 75-g oral glucose tolerance test (OGTT) during the pregnancy; venous plasma glucose ≥ 5.3 mmol/L when fasting, ≥ 10.0 mmol/L at 1 hour or ≥ 8.6 mmol/L at 2 hours. The diagnostic criteria of GDM were the same as in current Finnish guidelines [24].
– Control group: normal OGTT results during the pregnancy and birth weight of the newborn < 4.5 kg

Exclusion criteria were as follows:

– Multiple pregnancy
– Suspected or verified endocrine or malignant disease
– Treatment of or known clinical history of psychiatric illness
– Substance abuse
– GDM group: diagnosed type 1 or 2 diabetes before the index pregnancy
– Control group: GDM in earlier pregnancy

Resting blood pressure and heart rate, weight (kg), height (cm) and waist circumference (cm) of the participants were measured. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²). Metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP Adult Treatment Panel III) as the presence of at least three of the following five criteria [10]:

– waist circumference > 88 cm
– serum triglycerides ≥ 1.7 mmol/L
– serum high-density lipoprotein (HDL) cholesterol level < 1.3 mmol/L
– blood pressure ≥ 130/85 mmHg
– plasma glucose level ≥ 6.1 mmol/L or diabetes mellitus

Further, we interviewed the participants as regards their medical histories and lifestyle habits. Initially successful weight loss followed by weight regain (so called “yo-yo” dieting or weight cycling) is associated with body weight excess and abdominal fat accumulation [25]. To analyse “yo-yo” dieting, we estimated total lifetime weight loss by adding together kilograms lost during every previous intentional weight-loss period. Lifetime tobacco exposure was calculated as pack-years by multiplying smoking years with average packs smoked daily [26]. One pack-year is defined as twenty cigarettes smoked every day for one year.

![Figure 1](image1.png)

**Figure 1** Pack-years of smoking in the GDM and control groups. Pack-years of smoking differed significantly (p = 0.012) between women with a previous history of GDM vs. women unaffected. The median in both groups was zero, because the majority were non-smokers. The mean (±SD) number of pack-years in the GDM group was 3.1 (±6.1) and in the control group, 1.6 (±4.4).
The primary outcome was to define the prevalence of MetS and its different variables in the GDM and control groups. We wanted to see if there were differences in medical history, lifestyle habits, pregnancy outcomes or clinical characteristics between the groups. The secondary aim was to investigate the influence of excess body weight on these results.

Every participant was given both oral and written information on the study before she signed an informed consent document. The study protocol was approved by the Ethics Committee of Kanta-Häme Hospital District and the study followed the ethical principles outlined in the Declaration of Helsinki [27].

Basic blood count and serum levels of creatinine, alanine transaminase (ALAT), fasting glucose, total cholesterol, HDL cholesterol, low-density-lipoprotein (LDL) cholesterol and triglycerides, and the urinary albumin to creatinine ratio, as well as fibrinogen, were analysed according to validated methods after at least 12 hours of fasting. Direct analyses of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were carried out by using commercial reagents from Beckman Coulter (Brea, CA, USA). Analyses of ALAT (IFCC method), creatinine (Jaffé method) and plasma glucose (hexokinase method) were carried out by using commercial reagents from Beckman Coulter, with an Olympus AU640 analyser and analyses of fibrinogen (Clauss method) by using Siemens BCS XP equipment.

Table 3 Laboratory characteristics of participants with GDM vs. controls

<table>
<thead>
<tr>
<th></th>
<th>GDM</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 119)</td>
<td>(n = 120)</td>
<td></td>
</tr>
<tr>
<td>Leucocytes, 10^9/L</td>
<td>5.8 ± 1.6</td>
<td>5.2 ± 1.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Haemoglobin, g/L</td>
<td>133.2 ± 9.3</td>
<td>128.6 ± 12.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelets, 10^9/L</td>
<td>241.9 ± 58.2</td>
<td>244.0 ± 52.5</td>
<td>NS</td>
</tr>
<tr>
<td>ALAT, U/L</td>
<td>22.8 ± 17.4</td>
<td>19.7 ± 10.5</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, umol/L</td>
<td>66.6 ± 7.7</td>
<td>64.5 ± 7.8</td>
<td>0.048</td>
</tr>
<tr>
<td>U-AlbCre, mg/mmol</td>
<td>0.67 ± 0.5</td>
<td>0.57 ± 0.3</td>
<td>NS (0.070)</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.4 ± 0.9</td>
<td>3.2 ± 1.0</td>
<td>NS (0.096)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.6 ± 0.6</td>
<td>5.3 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.7 ± 0.9</td>
<td>4.6 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.5 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.0 ± 0.7</td>
<td>2.8 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.1 ± 0.6</td>
<td>0.9 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
U-AlbCre: urinary albumin to creatinine ratio, ALAT: alanine transaminase.

Table 4 Clinical characteristics of non-obese GDM cases and their controls, and obese GDM cases and their controls

<table>
<thead>
<tr>
<th></th>
<th>GDM cases</th>
<th>Controls</th>
<th>Overall p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMI ≥ 27 (n = 65)</td>
<td>BMI &lt; 27 (n = 55)</td>
<td>BMI ≥ 27 (n = 57)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg*</td>
<td>126.6 ± 12.3</td>
<td>117.7 ± 11.2</td>
<td>122.8 ± 12.4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg*</td>
<td>76.1 ± 9.6</td>
<td>70.5 ± 9.6</td>
<td>74.6 ± 8.1</td>
</tr>
<tr>
<td>Mean peripheral pressure, mmHg*</td>
<td>94.0 ± 10.7</td>
<td>87.0 ± 8.4</td>
<td>91.5 ± 9.3</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>66.6 ± 8.9</td>
<td>65.2 ± 9.3</td>
<td>65.2 ± 9.0</td>
</tr>
<tr>
<td>MetS, n (%)</td>
<td>15 (23.1 %)</td>
<td>4 (3.3 %)</td>
<td>8 (14.0 %)</td>
</tr>
<tr>
<td>- Waist circumference &gt; 88 cm, n (%)</td>
<td>62 (95.4 %)</td>
<td>27 (49.1 %)</td>
<td>53 (93.0 %)</td>
</tr>
<tr>
<td>- Blood pressure ≥ 130/85 mmHg, n (%)</td>
<td>27 (41.5 %)</td>
<td>8 (14.5 %)</td>
<td>19 (33.3 %)</td>
</tr>
<tr>
<td>- HDL cholesterol &lt; 1.30 mmol/L, n (%)</td>
<td>14 (21.5 %)</td>
<td>9 (16.4 %)</td>
<td>14 (24.6 %)</td>
</tr>
<tr>
<td>- Triglycerides ≥ 1.7 mmol/L, n (%)</td>
<td>9 (13.8 %)</td>
<td>3 (5.5 %)</td>
<td>4 (7.0 %)</td>
</tr>
<tr>
<td>- Glucose ≥ 6.1 mmol/L or diabetes, n (%)</td>
<td>11 (16.9 %)</td>
<td>7 (12.7%)</td>
<td>1 (1.8 %)</td>
</tr>
</tbody>
</table>

Metabolic syndrome and separate variables defined by NCEP.
Data are presented as mean ± SD if not mentioned otherwise.
*Differences between non-obese GDM cases and their controls, and obese GDM cases and their controls were non-significant; differences in other subgroup comparisons were significant.
Figure 2 (See legend on next page.)
relatively well represents average BMI among Finnish women [28]. Medicines agencies also define the cut-off point of overweight as a BMI of 27 kg/m² [29]. There were 122 women in the “obese” group (BMI ≥ 27); 65 GDM and 57 control participants. The “non-obese” group (BMI < 27; n = 118) consisted of 55 GDM and 63 control participants. The clinical characteristics of these four subgroups were studied by way of one-way ANOVA in cases of normality and by using the Kruskal–Wallis test in cases of non-normality. Post hoc analyses were performed, when appropriate. Logistic regression analysis was carried out to identify predictors as regards the presentation of MetS. First, univariate analysis was carried out. The set of independent variables tested included previous GDM, maternal age, BMI, family history of diabetes mellitus, pack-years of smoking, total lifetime weight loss, method of treatment among GDM cases, birth weight of the newborn, time from delivery to the present study and serum concentration of total cholesterol. The significant independent variables were then entered into multivariate analysis. The results are expressed as odds ratios (ORs) and 95% confidence intervals (CIs). A two-tailed probability value of < 0.05 was considered significant.

Results

Basic information on the index pregnancy in the GDM and control groups is shown in Table 1. All GDM participants and controls underwent a 75-g OGTT during the index pregnancy. A total of 25 GDM participants had medication during their pregnancies (insulin, n = 24; metformin, n = 1), while the other mothers in the GDM group had only dietary therapy. Twenty-three of the 120 women were primiparous in both groups. Nearly a third (29.9%, n = 29/97) of the multiparous GDM participants had already experienced GDM in an earlier pregnancy. Accumulation of gestational hypertension and pre-eclampsia was more common in diabetic pregnancies (p = 0.038). There was more glucosuria and proteinuria in pregnancies affected by GDM, as shown in Table 1.

The average time to follow-up was 3.7 years in both study groups. Clinical characteristics in women with and without previous GDM are shown in Table 2. According to our study interview data there were more current or former smokers in the GDM group than in the control group, and also the pack-years of smoking differed significantly (Figure 1). The groups did not differ in physical activity, alcohol intake or lifetime weight loss. The GDM group used less margarine weekly than the control group (n = 64 vs. 81; p = 0.034), but on the other hand the groups did not differ in weekly use of butter (n = 69 vs. 66). The GDM participants also consumed fewer sweets and sweet baked goods weekly (n = 95 vs. 111; p = 0.005) than the controls. Otherwise, we found no other differences in basic nutrition habits between the groups.

Despite a current Finnish guideline recommending OGTT screening six to twelve weeks after delivery in cases of medicated GDM during pregnancy, and one year after delivery in diet-treated GDM during pregnancy [24], only 41 of the 120 women (34.2%) with a history of GDM had an OGTT after delivery. Of these, 39.0% (16/41) showed glucose intolerance as follows: 17.1% (7/41) had impaired fasting glucose (IFG), 14.6% (6/41) had impaired glucose tolerance (IGT) and 7.3% (3/41) had diabetes. The results of OGTTs were normal in 25 of the 41 cases.

Clinical chemical data concerning the women with and without previous GDM are presented in Table 3. Between the groups, there were significant differences in serum concentrations of fasting glucose and triglycerides, both of them variables of MetS. When GDM participants with medication (n = 25) were compared with those with dietary therapy (n = 95) during the index pregnancy, we noticed a significant difference only in fasting glucose (6.0 ± 1.0 vs. 5.5 ± 0.4 mmol/L; p = 0.003). As shown in Table 2, the women in the GDM group met the criteria of MetS 2.4-fold more often than the controls. The numbers of participants with separate variables of metabolic syndrome defined by NCEP are also shown in Table 2.

In subgroup analyses, MetS affected participants in obese subgroups more often than in non-obese subgroups, as shown in Table 4. These four subgroups, obese GDM cases and their controls, and non-obese GDM cases and their controls, did not differ significantly in family history of cardio- or cerebrovascular diseases, medical history, medication, contraception, physical activity or alcohol consumption. Pack-years of smoking among non-obese GDM women were 2.7 (±3.5), among obese GDM women 4.7 (±7.5), among non-obese
Figure 3 (See legend on next page.)
control women 1.6 (±3.5) and among obese control women 3.3 (±5.5) (p = 0.058). The subgroups did not differ significantly in perinatal outcomes either. There was a major difference in lifetime weight loss (Figure 2A), both obese GDM and obese control women having lost more weight than non-obese GDM and control women. There were differences in most of the basic clinical characteristics between these four subgroups, particularly between non-obese and obese subgroups, as demonstrated in Figures 2B, C and 3A–C, and Table 4.

In univariate logistic regression analysis, previous GDM (OR 2.63, 95% CI 1.11–6.28; p = 0.029), higher BMI values (OR 1.24, 95% CI 1.14–1.35; p < 0.001), greater lifetime weight loss (OR 1.02, 95% CI 1.00–1.03; p = 0.013) and higher levels of total cholesterol (OR 1.26–3.10; p = 0.003) were associated with an increased risk of MetS. Multivariate analysis also showed that previous GDM (OR 2.83, 95% CI 1.05–7.63; p = 0.040), higher BMI values (OR 1.24, 95% CI 1.13–1.36; p < 0.001) and higher serum concentrations of total cholesterol (OR 1.68, 95% CI 1.01–2.79; p = 0.046) seemed to predict the presentation of MetS. No other associations were found in logistic regression analyses.

**Discussion**

The main finding in our study was that the risk of developing MetS after GDM was 2.4-fold greater than after normal pregnancy. However, cardiovascular risk factors such as increased LDL cholesterol and triglyceride levels as well as decreased HDL cholesterol concentrations were more common in participants with high BMI than in those with previous GDM.

A systematic review conducted in 2014 demonstrated that women who have had GDM have a nearly fourfold increased risk of developing MetS in the future than those who have had a normal pregnancy. However, there are some factors that may modify the risk of developing MetS after GDM. For example, ethnicity may significantly affect MetS susceptibility. BMI is also an important confounder in the overall MetS risk estimate. When MetS after GDM was grouped by BMI, the odds ratio was 2.53 according to recent meta-analyses [6]. In our study, both the participants and the controls were of Caucasian origin, and there was no significant difference between the groups in BMI or body weight. Our results are in accordance with results reported earlier [6].

The results of previous studies indicate that there is a relationship among the risk gene variants as regards both GDM and MetS [30-32]. Possibly, genetic factors also protect obese control women against insulin resistance and, on the other hand, expose non-obese or even lean GDM women to glucose intolerance during pregnancy. At the same time, non-obese GDM women seem to have a better cardiovascular profile a few years after their index pregnancies than both obese groups. Cross-sectional analysis of different variables does not foretell the prognosis of women in the future. According to our results, obesity seems to represent a greater risk of MetS and presentation of cardiovascular risk variables than previous GDM, at least after a few years of delivery. The results of multivariate analysis supported the main findings.

A strength of our study is that all participants had undergone OGTT screening during the index pregnancy. In Finland, GDM screening via 75-g OGTTs is offered to all pregnant women at risk of GDM. Current care guidelines in Finland do not recommend OGTT screening for low-risk women – primiparous women < 25 years old, BMI ≤ 25 kg/m², and no family history of DM, or multiparous women < 40 years old, no GDM in previous pregnancy or pregnancies, and BMI ≤ 25 kg/m² before the current pregnancy [24].

OGTT screening has been carried out in 51.5% of pregnancies during the past five years in our area. We wanted to be sure that the controls really were unaffected as regards glucose intolerance and had undergone OGTTs during their index pregnancies. This situation could reflect a hidden weakness of our study, since maybe the best controls, being part of the 48.5% low-risk parturients who did not undergo OGTT screening during pregnancy, were excluded from the study. Another ambiguous matter was the BMI cut-off point of 27 kg/m², because obesity is commonly classified as BMI ≥ 30 kg/m² [33]. In our subgroup analysis, we used BMI to divide our study group into two halves, intending to reveal the effect of excess body weight on cardiovascular risk factors. According to the FINRISK 2012...
Study, mean BMI among women aged 25–74 years is 26.8 kg/m² in Finland [28], so actually our cut-off point of BMI fairly well represents average BMI among Finnish women. Medicines agencies in Europe and in the USA define the cut-off point of overweight as a BMI of 27 kg/m². Arguments for this definition have been discussed in detail earlier [29].

Women who have had GDM are advised to have glucose tolerance assessed postpartum [24,34]. The low rate of attendance at follow-up suggests that many healthcare providers may not recognize GDM as an initial warning sign of predisposition to MetS. In Finland, there is no consensus of opinion regarding how to monitor obese women after normal pregnancy, but according to our results, we suggest that unaffected obese women should undergo screening for at least cardiovascular risk factors after delivery. Paying attention to patients with pathological OGTT results as well as an overweight condition during and after pregnancy helps healthcare professionals to identify women who may be at risk of developing MetS.

Conclusions
In conclusion, the risk of metabolic syndrome was 2.4 times higher after GDM compared with normoglycaemic pregnancy, but the risk factors of coronary heart disease were even more evident in women with excess body weight. Women with previous GDM, particularly obese ones, and also unaffected obese women should not miss the opportunity to prevent future metabolic disease.

Abbreviations
ALAT: Alanine transaminase; BMI: Body mass index; GDM: Gestational diabetes mellitus; HDL: High density Lipoprotein; LDL: Low density Lipoprotein; MetS: Metabolic syndrome; NCEP: National cholesterol education program; OGTT: Oral glucose tolerance test; OR: Odds ratio; CI: Confidence interval.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
TV-K, MV and AP designed the study; TV-K conducted experiments and performed data analysis with the help of AP; TV-K, OP and AP drafted the manuscript; all authors critically revised the manuscript, and, finally, read and approved the manuscript.

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References


Original Research Article

Oxidized LDL, insulin resistance and central blood pressure after gestational diabetes mellitus

Running headline: Cardiovascular risk factors after GDM

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Abstract

**Introduction:** Gestational diabetes mellitus (GDM) is an indicator of future cardiovascular disease. We investigated if sensitive biomarkers of increased cardiovascular risk differ between women with and without a history of GDM few years after pregnancy, and whether obesity affects the results.

**Material and methods:** We studied two cohorts – 120 women with a history of GDM and 120 controls, on average 3.7 years after delivery. Circulating concentrations of oxidized low-density lipoprotein (oxLDL) were determined by ELISA. The homeostasis model assessment of insulin resistance (HOMA-IR) index was used to estimate insulin resistance. Central blood pressure (cBP) was measured noninvasively from a radial artery pulse wave. The primary outcomes were possible differences in oxLDL, HOMA-IR or cBP between the groups. Secondly, we investigated the influence of obesity on the results, also by using adjusted multiple linear regression analyses.

**Results:** OxLDL concentrations or cBP did not differ between the two cohorts, but HOMA-IR was significantly higher in women with previous GDM than in controls, 1.3 ± 0.9 (SD) and 1.1 ± 0.9 respectively (p = 0.022). In subgroup analyses, HOMA-IR (p < 0.001), systolic (p < 0.001) and diastolic (p < 0.001) cBP were significantly higher in obese subgroups compared with non-obese ones. Body mass index (BMI) was an important determinant of HOMA-IR and cBP in multiple linear regression analyses.

**Conclusions:** Over three years after delivery women with GDM were still more insulin resistant than controls. Obesity turned out to be a more important determinant of insulin resistance and cBP than GDM.
Keywords: central blood pressure, gestational diabetes mellitus, homeostasis model assessment of insulin resistance, obesity, oxidized low-density lipoprotein

Abbreviations: BMI: body mass index; cBP: central blood pressure; GDM: gestational diabetes mellitus; HOMA-IR: homeostasis model assessment of insulin resistance; OGTT: oral glucose tolerance test; oxLDL: oxidized low-density lipoprotein

Key message

Biomarkers reflecting increased cardiovascular risk were revealed in women with obesity or previous gestational diabetes mellitus already few years after pregnancy. Obesity may be an even more important determinant of insulin resistance and central blood pressure than previous gestational diabetes.
Introduction

Gestational diabetes mellitus (GDM) is the most common metabolic complication of pregnancy, and its global prevalence is approximately 7% varying from one to 14 percent depending on diagnostic tests and the population studied (1). In Finland, GDM was found in 15.9% of pregnancies in 2014 (2). The relatively high percentage of GDM in Finland might – at least partly – be explained by an extensive screening program, according to national guidelines (3). GDM has significant implications for the future health of the mother. For instance, it is associated with increased insulin resistance and risk of type 2 diabetes (4), which are known to be involved in the atherosclerotic process (5).

Atherosclerosis begins with accumulation of lipoproteins, particularly low-density lipoprotein (LDL), in the intima of arteries. In the arterial wall, LDL particles undergo oxidative modification, which plays an important role in the atherosclerotic process (6). Circulating oxidized LDL (oxLDL) seems to reflect the level of oxidative stress (7). Further, increased amounts of circulating oxLDL are associated with the occurrence of coronary heart disease (8, 9). There is accumulating evidence that type 2 diabetes is associated with increased oxidative stress (10, 11), but there are no earlier studies on oxLDL levels after GDM.

The prognostic value of brachial blood pressure is well known (12). However, noninvasively determined central blood pressure (cBP) seems to be even more relevant than peripheral pressure as regards the pathogenesis of cardiovascular disease (13, 14). cBP also correlates with cardiovascular risk in seemingly healthy subjects (12).

As the prevalence of GDM has increased rapidly in recent decades (15), better understanding of the connections between previous GDM and cardiovascular risk factors would be of great value. Our primary aim was to study whether or not concentrations of circulating oxLDL, insulin resistance determined by the homeostasis model assessment of insulin resistance (HOMA-IR) index or cBP could reveal an elevated cardiovascular risk already as early as a few years after GDM. The secondary aim was to investigate the influence of obesity on the results.

Material and methods

In this follow-up study of 240 women aged 35.8 ± 4.5 (SD; range 25–46) years, a total of 120 women with a history of GDM during the index pregnancy were compared with 120 age-matched women with normal glucose metabolism during pregnancy. The control group was
also matched according to the time interval from index pregnancy to follow-up study. All subjects had delivered 2–6 years earlier at Kanta-Häme Central Hospital, Finland, i.e. after the publication of Finnish Current Guidelines for screening GDM. The inclusion and exclusion criteria with power analysis have been described earlier (16). Briefly, GDM was defined as any pathological value in a 2-h 75-g oral glucose tolerance test (OGTT) during pregnancy (venous plasma glucose ≥ 5.3 mmol/L when fasting, ≥ 10.0 mmol/L at one hour or ≥ 8.6 mmol/L at two hours). The diagnostic criteria of GDM were the same as in Finnish Current Guidelines, which were published in 2008 and updated 2013 without any change in the diagnostic criteria of GDM (2, 3). Thus, every GDM patient in our study was diagnosed according to uniform criteria. Our national diagnostic cut points of GDM are quite similar to those of the International Association of the Diabetes and Pregnancy Study Groups (IADPSG), in which the threshold values of plasma glucose are ≥ 5.1 mmol/L when fasting, ≥ 10.0 mmol/L at one hour or ≥ 8.5 mmol/L at two hours (17). Only singleton pregnancies were accepted. Women were excluded if they had suspected or verified malignant or endocrine disease, diagnosed type 1 or 2 diabetes before the pregnancy, substance abuse or treatment, a known clinical history of psychiatric illness or if they were pregnant at time of the study. Controls without GDM had to have had normal OGTT results during the pregnancy and the weight of the newborn had to be less than 4.5 kg. Controls without GDM were excluded if they had experienced GDM in an earlier pregnancy. In Finland GDM screening using a 75 g OGTT is offered to all pregnant women, except those who are at the lowest risk: primiparous women < 25 years old, BMI ≤ 25 kg/m2 and no family history of DM, or multiparous women < 40 years old, no GDM in previous pregnancy or pregnancies and BMI ≤ 25 kg/m2 before the current pregnancy. The electronic database of the hospital was used to pick up the participants for both cohorts. Both recruitment and examinations were carried out between August 2011 and July 2014.

Resting heart rate, weight (kg) and height (cm) of the participants were measured. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²). Further, we interviewed the participants as regards their medical histories and lifestyle habits. Although we did not try to standardize the study groups according to exercise, interview on physical activity did not reveal differences between groups (16).

Two experienced nurses measured cBP after at least ten minutes of rest in a prone position. It was estimated non-invasively from a radial artery pulse wave by way of a SphygmoCor device (AtCor Medical, Sydney, Australia), which uses radial pulse and a validated
generalized transfer function to estimate central pressures from brachial BP and peripheral pulse waves (12). The participants were asked to refrain from eating, drinking caffeinated drinks, smoking and taking medication for 12 hours, and drinking alcohol for two days prior to measurement. Three measurements were performed to obtain mean cBPs for every participant. Values of cBP are indirect surrogate measures of arterial stiffness, but they provide additional information concerning pulse wave reflections (18).

Every participant was given both oral and written information on the study before she signed an informed consent document. The study protocol was approved by the Ethics Committee of Kanta-Häme Hospital District (reference number 521/2010; date of approval 21.12.2010) and the study followed the ethical principles outlined in the Declaration of Helsinki (19).

Plasma concentrations of oxLDL were determined by using a validated ELISA method (Mercodia AB, Uppsala, Sweden). The assay kits include the same monoclonal antibody (4E6) as originally described by Holvoet et al. (8, 9). An Evolis ELISA analyzer (Bio-Rad, Marnes-la-Coquette, France) was used to run the assays. Plasma levels of oxLDL were determined by comparison with standards included in each assay. The results were expressed as units per liter (U/L). The total coefficient of variation of the assay (including both interassay and intra-assay variability) was 8.5%.

Fasting levels of plasma glucose and insulin were analyzed according to validated methods. Assay of plasma glucose was carried out by using a standardized hexokinase method and that of glycosylated hemoglobin (HbA1c) by way of a standardized immunochemical method with commercial reagents from Beckman Coulter and an Olympus AU640 analyzer. Insulin levels were measured by electrochemiluminescence immunoassay (ECLIA) (Roche Cobas, Basel, Switzerland). According to the International Expert Committee (IEC) 2009 criteria, glycemic categories were based on the following HbA1c cut points: normal, HbA1c < 42 mmol/mol; prediabetes, HbA1c ≥ 42 mmol/mol but < 48 mmol/mol; and diabetes, HbA1c ≥ 48 mmol/mol (20). The homeostasis model assessment of insulin resistance (HOMA-IR) index is based on a single measurement of plasma glucose and insulin and is commonly used as a parameter of the severity of insulin resistance (21). It was calculated thus: fasting insulin (mU/L) × fasting blood glucose (mmol/L)/22.5 (22). Routine laboratory analyses were examined according to validated methods as described in detail earlier (16).
Statistical analysis

Statistical analysis was carried out by using IBM® SPSS® Statistics Version 22 software (copyright 2013). Variables were tested for normality by way of Shapiro–Wilk or Kolmogorov–Smirnov tests, as appropriate. Data are presented as mean ± standard deviation (SD) if not mentioned otherwise. Differences in continuous variables between GDM participants and controls were studied by using Student's t-test in cases of normality and by the Mann–Whitney U-test in cases of non-normality. Further, we analyzed whether drug therapy of GDM during the pregnancy affected the primary outcome. To study the effect of obesity on the results, we divided the whole study group into four subgroups according to obesity and previous GDM. Obesity was classified as BMI of ≥ 30 kg/m² (23). The clinical characteristics of the subgroups were studied by one-way ANOVA in cases of normality and by using the Kruskal–Wallis test in cases of non-normality. Post hoc analyses were performed by using Fisher's least significant difference method for multiple comparisons, when appropriate. If overall p value was significant, individual p values between subgroups were also presented. Multiple linear regression analyses were conducted to examine whether simple associations were changed after adjustment for potential confounders. We selected clinically relevant covariates in the multiple-adjusted models including age, BMI, previous GDM, total cholesterol, high-density lipoprotein cholesterol, fasting glucose, heart rate, ALAT and smoking status. A two-tailed probability value of < 0.05 was considered significant.

Results

The basic clinical characteristics of the study groups are summarized in Table 1. Plasma levels of HbA1c were higher in the GDM group, but there was no difference in plasma concentrations of fasting insulin. According to HbA1c (20), one participant had diabetes and four had prediabetes in the GDM group, while all the controls were in the normal glycemic category (p = 0.076).

There was no difference in plasma concentrations of oxLDL between women with GDM and controls. HOMA-IR index values were significantly higher in the GDM group. Differences in central systolic and diastolic pressure did not reach statistical significance. We found no difference in central mean pressure (90.7 ± 10.3 vs. 88.3 ± 9.5 mmHg; p = 0.089) between the study groups (Table 2).
Table 1: The basic clinical characteristics of GDM women and controls. Data are presented as mean ± SD if not mentioned otherwise.

<table>
<thead>
<tr>
<th></th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average time from delivery, years</td>
<td>3.7 ± 1.0</td>
<td>3.7 ± 0.9</td>
<td>0.818</td>
</tr>
<tr>
<td>Age, years</td>
<td>35.8 ± 4.4</td>
<td>35.9 ± 4.6</td>
<td>0.854</td>
</tr>
<tr>
<td>Primiparous, n (%)</td>
<td>23 (19.2%)</td>
<td>23 (19.2%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Therapy of GDM during the pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- insulin, n (%)</td>
<td>24 (20.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- metformin, n (%)</td>
<td>1 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- dietary therapy, n (%)</td>
<td>95 (79.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pack years of smoking</td>
<td>3.8 ± 6.0</td>
<td>2.4 ± 4.6</td>
<td>0.012</td>
</tr>
<tr>
<td>BMI, kg/m^2</td>
<td>28.3 ± 5.0</td>
<td>27.5 ± 5.4</td>
<td>0.069</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>122.4 ± 12.5</td>
<td>119.0 ± 11.5</td>
<td>0.034</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73.5 ± 9.0</td>
<td>71.8 ± 8.7</td>
<td>0.176</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>65.9 ± 9.1</td>
<td>63.8 ± 9.6</td>
<td>0.017</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.7 ± 0.9</td>
<td>4.6 ± 0.8</td>
<td>0.329</td>
</tr>
<tr>
<td>ALAT, U/L</td>
<td>22.8 ± 17.4</td>
<td>19.7 ± 10.5</td>
<td>0.116</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.6 ± 0.6</td>
<td>5.3 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HbA1c, mU/L</td>
<td>34.9 ± 3.3</td>
<td>33.8 ± 1.8</td>
<td>0.012</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>5.2 ± 3.6</td>
<td>4.6 ± 3.6</td>
<td>0.087</td>
</tr>
</tbody>
</table>

ALAT: alanine transaminase; BMI: body mass index; BP: blood pressure; HbA1c: hemoglobin A1c

During the pregnancy insulin or metformin -treated women with GDM (n = 25), dietary treated women with GDM (n = 95) and controls (n = 120) were compared by variables of primary outcome, we noticed a significant difference in HOMA-IR (p = 0.016). HOMA-IR was among medicated GDM participants 1.6 ± 1.3, among dietary treated GDM participants 1.2 ± 0.8 and among controls 1.1 ± 0.8 (p = 0.034 against medicated GDM). No differences were noticed in the values of systolic or diastolic cBP or oxLDL between the medicated and dietary treated GDM participants or controls.
Table 2. Primary analysis of GDM and control groups. Data are presented as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>GDM</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxLDL, U/L</td>
<td>42.4 ± 14.4</td>
<td>39.7 ± 13.8</td>
<td>0.120</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.3 ± 0.9</td>
<td>1.1 ± 0.9</td>
<td>0.022</td>
</tr>
<tr>
<td>Systolic cBP, mmHg</td>
<td>110.6 ± 12.4</td>
<td>107.5 ± 11.5</td>
<td>0.061</td>
</tr>
<tr>
<td>Diastolic cBP, mmHg</td>
<td>74.5 ± 9.1</td>
<td>72.7 ± 8.8</td>
<td>0.123</td>
</tr>
</tbody>
</table>

cBP: central blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance; oxLDL: plasma concentration of oxidized low-density lipoprotein

In subgroup analyses, there were 75 women in the obese group (BMI ≥ 30 kg/m²), i.e. 43 GDM and 32 control participants. The non-obese group (BMI < 30 kg/m²; n=165) consisted of 77 GDM and 88 control participants. These four subgroups, obese women with GDM and their controls, and non-obese women with GDM and their controls, did not differ as regards circulating oxLDL levels (Figure 1). There were significant differences in plasma concentrations of fasting glucose and insulin, and also in HOMA-IR index values, as illustrated in Figure 2. The highest levels of fasting insulin were in the obese control group. Both systolic and diastolic cBP differed significantly in the four subgroups (Figure 3).
Median (minimum, maximum) levels of plasma oxLDL: among obese GDM women, 46 (19, 89) U/L, obese control women, 41 (24, 95) U/L, non-obese GDM women, 38 (18, 93) U/L, and non-obese control women, 36 (16, 99) U/L. Overall p value is given.

The results of multiple linear analyses are shown in Table 3. In multiple-adjusted models, BMI was a significant determinant of the HOMA-IR index, and systolic and diastolic cBP, but it was not associated significantly with plasma levels of oxLDL. In contrast, previous GDM was not an important influencing factor as regards any of the primary outcome measurements. Covariates of each parameter explained 39.8% of oxLDL, 34.6% of HOMA-IR, 23.2% of systolic cBP and 22.7% of diastolic cBP (Table 3).
Figure 2 Fasting glucose (A), insulin (B) and HOMA-IR (C) in the four subgroups.

A: Median (minimum, maximum) levels of fasting plasma glucose: among obese GDM women, 5.7 (4.9, 6.8) mmol/L, obese control women, 5.3 (4.4, 6.2) mmol/L, non-obese GDM women, 5.4 (4.6, 9.8) mmol/L, and non-obese control women, 5.2 (4.5, 6.1) mmol/L. B: Median (minimum, maximum) levels of fasting plasma insulin: among obese GDM women, 5.4 (0.6, 23.2) mmol/L, obese control women 6.4, (2.2, 23.1) mmol/L, non-obese GDM women, 3.8 (0.8, 16.6) mmol/L, and non-obese control women, 3.3 (0.4, 10.3) mmol/L. C: Median (minimum, maximum) HOMA-IR index values: among obese GDM women, 1.4 (0.2, 5.7), obese control women, 1.5 (0.5, 6.4), non-obese GDM women, 0.9 (0.2, 3.6), and non-obese control women, 0.8 (0.1, 2.4). Overall p value is given in the bottom. Individual p values for pairwise comparisons are also presented.
Figure 3 Central systolic (A) and diastolic pressures (B) in the four subgroups.

A: Median (minimum, maximum) central systolic pressure: among obese GDM women, 115 (97, 154) mmHg, obese control women, 111 (96, 161) mmHg, non-obese GDM women, 105 (90, 146) mmHg, and non-obese control women, 104 (84, 140) mmHg. B: Median (minimum, maximum) central diastolic pressure: among obese GDM women, 79 (61, 106) mmHg, obese control women, 76 (63, 91) mmHg, non-obese GDM women, 71 (55, 91) mmHg, and non-obese control women, 70 (54, 94) mmHg. Overall p value is given in the bottom. Individual p values for pairwise comparisons are also presented.
Table 3. Results of stepwise multiple linear regression analyses. Covariates in the multiple-adjusted analyses included age, BMI, previous GDM, TC, HDL-C, fasting glucose, heart rate, ALAT and smoking status. Final models include significant covariates only. Standardized $\beta$ provides a measure of the relative strength of an association, independent of the measurement units. Standardized $\beta$ and $p$ values are shown only when $p < 0.05$.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Covariates included in the model</th>
<th>$R^2$ for model</th>
<th>Global $p$</th>
<th>Standardized $\beta$</th>
<th>$p$ value</th>
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<tr>
<td></td>
<td>HDL-C</td>
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<td>0.346</td>
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<td>0.394</td>
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<td>HDL-C</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
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<td>0.009</td>
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<tr>
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<td>0.417</td>
<td>$&lt; 0.001$</td>
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<tr>
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<tr>
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<td>0.375</td>
<td>$&lt; 0.001$</td>
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<tr>
<td></td>
<td>Age</td>
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<td>0.180</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td></td>
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</tbody>
</table>

ALAT: alanine transaminase; BMI: body mass index; cBP: central blood pressure; HDL-C: low-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; oXLDL: oxidized low-density lipoprotein; TC: total cholesterol

Discussion

We found no significant differences in oXLDL or cBP measurements after GDM compared with normoglycemic pregnancy, but women with GDM were more insulin resistant than those without. Obesity turned out to be a more important determinant of insulin resistance and cBP than GDM.
Oxidized LDL, when accumulating in the arterial wall, injures its endothelium, leading to endothelial dysfunction (6). Endothelial dysfunction leads to impaired arterial elasticity at an early stage in the atherosclerotic process (24). Previously it has been shown that circulating oxLDL levels are significantly higher among men with metabolic syndrome than among controls (25). However, a search of MEDLINE (English language; 1961–June 2016; search terms: “oxLDL” and “GDM”) revealed no publications concerning female population where circulating oxLDL has been studied in connection with GDM. Since low-risk parturients do not undergo OGTTs in Finland, the healthiest subjects were not included in our study (16). Therefore, our non-significant findings concerning oxLDL and other variables may be compromised.

Glucose tolerance often normalizes after pregnancy complicated by GDM. However, previous investigators have proposed that glucose intolerance is frequent in the early postpartum period and these women have lower insulin sensitivity (26). The HOMA-IR index is a robust tool for the surrogate assessment of insulin resistance (27, 28), and it has also been proved to correlate with direct measurement of insulin sensitivity using the insulin clamp (21). Although the HOMA-IR method is mainly used to measure insulin sensitivity in large epidemiologic studies, we found a significant difference in HOMA-IR values between the study groups in our smaller study. The HOMA-IR results after GDM are in accordance with findings reported earlier (29).

One could presume that fasting insulin were higher in obese GDM than in obese control group. In subgroup analyses, however, the obese control group seemed to have the highest plasma concentrations of fasting insulin and the highest HOMA-IR index values, although their circulating concentrations of fasting glucose were significantly lower than in both of the GDM groups. GDM places affected women at a sevenfold risk of developing type 2 diabetes mellitus (4), so we may assume that some of our GDM women already have prediabetes. If so, their β-cell function may already be impaired, leading to decreased levels of fasting insulin. Multiple regression analyses of our data highlighted the association between BMI and HOMA-IR. This emphasizes the necessity of counseling a healthy lifestyle among women with obesity or previous GDM in order to prevent complications of cardiovascular diseases and decrease the burden of developing type 2 diabetes mellitus in the future.

As well as oxLDL, increased cBP has been independently associated with coronary artery disease (30, 31). CBP correlates to cardiovascular end points (13, 32, 33) and appears to
reflect cardiovascular risks earlier than brachial measurements (14). According to earlier studies, women with previous GDM have increased rates of selected cardiovascular risk factors (34, 35). Our previous and recent findings were partly in line with these results (16). Although increased BMI was associated with higher cBP in subgroup and multiple linear regression analyses, women with previous GDM did not differ from control group in results of cBP. Because our primary aim was to compare women with and without previous GDM already a few years after delivery, it is possible that upcoming differences in risk markers are not yet evident in our study.

Strength of our study is that the measurement methods are internationally widely used and well validated (8, 9, 13). Further, the study cohorts were well matched according to age and time between delivery and the present study. All participants, including all parturients in the control group, had undergone OGTT screening during the index pregnancy. As mentioned earlier, this strength may also be a weakness, because women of the lowest risk were excluded. The estimation of insulin sensitivity using HOMA-IR is less precise than insulin clamp measurement, the gold standard for analyzing insulin resistance (21). However, HOMA-IR can give a good measure of insulin resistance.

Glucose metabolism differed in women with GDM and controls, but no significant differences were revealed in oxLDL or cBP measurements between the groups. The influence of obesity on the risk factors of coronary heart disease exceeded that of GDM. The prevalence of GDM is increasing rapidly along with obesity (2, 15). Women with previous GDM, particularly obese ones, but also unaffected obese women should not miss the opportunity to prevent future diabetes and cardiovascular disease by lifestyle intervention.

Acknowledgements

The authors thank Anna Silén, Taru Stranden and Nick Bolton for professional technical aid.

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References


Subclinical inflammation associated with prolonged TIMP-1 upregulation and arterial stiffness after gestational diabetes mellitus: a hospital-based cohort study

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Abstract

Background: Gestational diabetes mellitus (GDM) has significant implications for the future health of the mother. Some clinical studies have suggested subclinical inflammation and vascular dysfunction after GDM. We aimed to study whether concentrations of high-sensitivity C-reactive protein (hsCRP), tissue inhibitor of metalloproteinase-1 (TIMP-1), matrix metalloproteinase-8 (MMP-8) and -9, as well as values of arterial stiffness differ between women with and without a history of GDM a few years after delivery. We also investigated possible effects of obesity on the results.

Methods: We studied two cohorts—120 women with a history of GDM and 120 controls—on average 3.7 years after delivery. Serum concentrations of hsCRP were determined by immunonephelometric and immunoturbidimetric methods, MMP-8 by immunofluorometric assay, and MMP-9 and TIMP-1 by enzyme-linked immunosorbent assays. Pulse wave velocity (PWV) was determined using the foot-to-foot velocity method from carotid and femoral waveforms by using a SphygmoCor device. Arterial compliance was measured non-invasively by an HDI/PulseWave™CR-2000 arterial tonometer. All 240 women were also included in subgroup analyses to study the effect of obesity on the results. Multiple linear regression analyses were performed with adjustment for confounding factors.

Results: PWV after pregnancy complicated by GDM was significantly higher than after normal pregnancy, 6.44 ± 0.83 (SD) vs. 6.17 ± 0.74 m/s (p = 0.009). Previous GDM was also one of the significant determinants of PWV in multiple linear regression analyses. On the other hand, compliance indices of both large (p = 0.092) and small (p = 0.681) arteries did not differ between the study cohorts. Serum TIMP-1 levels were significantly increased after previous GDM (p = 0.020). However, no differences were found in the serum levels of MMP-8, MMP-9 or hsCRP. In subgroup analyses, there were significantly higher concentrations of hsCRP (p = 0.015) and higher PWV (p < 0.001) among obese women compared with non-obese ones.

Conclusions: PWV values were significantly higher after GDM compared with normoglycemic pregnancies and were associated with prolonged TIMP-1 upregulation. Cardiovascular risk factors were more common in participants with high BMI than in those with previous GDM.

Keywords: Arterial compliance, Gestational diabetes mellitus, High-sensitivity C-reactive protein, Matrix metalloproteinase-8, Matrix metalloproteinase-9, Pulse wave velocity, Subclinical inflammation, Tissue inhibitor of matrix metalloproteinase-1

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Background

In developed countries, the prevalence of gestational diabetes mellitus (GDM) has increased rapidly in recent decades, along with increasing rates of obesity [1, 2]. In Finland, GDM complicated 15.9% of pregnancies in 2015 [2]. A diagnosis of GDM has significant implications for the future health of the mother. For instance, GDM has been shown to be associated with postpartum insulin resistance, hypertension, and dyslipidemia [3–5], placing affected women at risk of metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM) and/or cardiovascular disease (CVD) later in life [5–8]. Incidence of CVD events, and specifically those of coronary artery disease, is known to be increased in women with previous GDM, even in the absence of T2DM [8]. Clinical studies have also revealed subclinical inflammation and vascular dysfunction after GDM [4].

High-sensitivity C-reactive protein (hsCRP) is a well-known acute-phase protein and a sensitive biomarker of systemic inflammation. Elevated levels of hsCRP are a significant risk factor for atherosclerosis [9]. The group of matrix metalloproteinases (MMPs) comprises over 20 structurally and functionally related but genetically distinct members [10, 11]. Expression and activity are normally low, but increased in many pathophysiological conditions. MMPs can modulate immunological responses, and MMPs can be either defensive or destructive [11]. Both upregulation and down-regulation of MMP-8 and -9 have been associated with several noninfectious as well infectious inflammatory states [12–18]. MMP-8 may also regulate blood pressure [19]. MMPs and their inhibitors, tissue inhibitors of MMPs (TIMPs) have been related to atherosclerosis development and progression in humans [20–22]. It has been suggested that imbalanced concentrations of MMP family members and TIMPs eventually exert an important role in cardiovascular risk [21–25].

Inflammation may be pathogenic, by inducing vascular dysfunction [4, 26]. Arterial stiffness has proven to be an important parameter for the assessment of cardiovascular risk, and it has earlier been associated with endothelial dysfunction [27, 28]. Carotid to femoral pulse wave velocity (PWV) has emerged as the gold standard to assess arterial stiffness [29]. When the arteries are stiff or less distensible, PWV increases [30, 31]. PWV increases proportionally to the number of cardiovascular risk factors present, such as diabetes or MetS [27, 32, 33]. In epidemiological studies, increased PWV has been predictive of cardiovascular events [29].

Recently, the implications of GDM as regards women’s future health have been widely discussed. As the prevalence of GDM has increased over the years, a better understanding of the connections between previous GDM and both subclinical inflammation and vascular dysfunction would be of great benefit. In addition, recently it has been suggested that MMP-8 is associated with insulin receptor degradation, and high serum MMP-8 levels with an increased risk of diabetes mellitus type II [17]. In previous studies serum levels of MMP-8, -9, TIMP-1 and hsCRP have been shown to be biomarkers reflecting low-grade inflammation [11, 23, 24, 34, 35]. In addition, TIMP-1 has been shown to exert MMP-independent actions such as pro-inflammatory and growth-factor-like properties [36–38].

With this background our aim was to define whether or not cardiovascular risk, assessed by serum concentrations of hsCRP, MMP-8, MMP-9 and TIMP-1, and values of arterial compliance and PWV are enhanced already a few years after GDM. We also evaluated the effect of obesity on the results.

Methods

In this follow-up study of two cohorts, a total of 120 women with a history of GDM during the index pregnancy were compared with 120 age-matched women with normal glucose metabolism during pregnancy. The time from the index pregnancy to the follow-up study was also matched between the study groups. All participants had delivered on average 3.7 (range 2–6) years earlier at Kanta-Häme Central Hospital, Finland, i.e. after the publication of Finnish Current Guidelines for screening GDM. Our national guidelines were published in 2008 and updated in 2013 without any change in the diagnostic criteria of GDM [39]. The complete inclusion and exclusion criteria, with power analysis, have been described earlier [40]. Briefly, GDM was defined (using the diagnostic criteria of Finnish Current Guidelines) as a pathological value in a 2-h 75-g oral glucose tolerance test (OGTT) during pregnancy: venous plasma glucose ≥5.3 mmol/L when fasting, ≥10.0 mmol/L at 1 h or ≥8.6 mmol/L at 2 h [39]. Our national diagnostic thresholds for GDM are similar to those of the International Association of Diabetes and Pregnancy Study Groups (IADPSG): plasma glucose ≥5.1 mmol/L when fasting, ≥10.0 mmol/L at 1 h or ≥8.5 mmol/L at 2 h [41]. Only singleton pregnancies were included. Women were excluded if they had type 1 or type 2 diabetes before the pregnancy, if they were pregnant at time of the study, if they had suspected or verified malignant or endocrine disease, if there was substance abuse or treatment, or a known clinical history of psychiatric illness. Controls had to have normal OGTT results during pregnancy. If the controls had experienced GDM in an earlier pregnancy, or the weight of the newborn was ≥4.5 kg, they were excluded. The electronic database of the hospital was used to pick up the cases and controls. Both recruitment...
and examinations were accomplished between August 2011 and July 2014. We interviewed the participants as regards their lifestyle habits. Lifetime tobacco exposure was estimated as pack-years, and one pack-year was defined as 20 cigarettes smoked every day for 1 year [42]. Further, we interviewed the participants as regards their history of trauma or infectious diseases during the previous month. We measured resting heart rate, brachial blood pressure, weight (kg) and height (cm) of the participants, and calculated body mass index (BMI): weight in kilograms divided by height in meters squared (kg/m²).

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki [43], and the protocol was approved by the Ethics Committee of Kanta-Häme Hospital District (reference number 521/2010; date of approval 21.12.2010). Every participant was given both oral and written information on the study before she signed an informed consent document.

**Laboratory methods**

Serum samples were collected after at least 12 h of fasting and stored at −80 °C until analyzed. Serum concentrations of hsCRP were analyzed according to validated immunonephelometric (United Medix Laboratories Ltd., Espoo, Finland) and immunoturbidimetric (VITA Healthcare Services Ltd., Vita Laboratory, Helsinki, Finland) methods [44, 45]. Concentrations of MMP-8 were determined by immunofluorometric assay (IFMA) (Medix Biochemica, Espoo, Finland), as previously described [25]. Serum levels of MMP-9 and TIMP-1 were analyzed by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Biotrak ELISA System; Amersham Biosciences, GE Healthcare, Buckinghamshire, UK) and according to the manufacturer’s instructions [18]. Fasting serum levels of total cholesterol (TC) and insulin were analyzed according to validated methods as described in detail earlier [40].

**Determination of arterial compliance and pulse wave velocity**

Three experienced nurses measured the compliance of large and small arteries after at least 10 min of rest in a semi-sitting position. The recording was carried out after an overnight fast. The participants were asked to refrain from eating, having caffeinated drinks, smoking and taking medication for 12 h, and drinking alcohol for 2 days prior to measurement. Radial artery pulse waves were recorded non-invasively with an arterial tonometer (HDI/PulseWave™ CR-2000, Hypertension Diagnostics, Inc., Eagan, Minnesota, USA) and the procedure involves the use of a modified Windkessel pulse-contour method [46]. Blood volume inertia and systemic vascular resistance are used to analyze arterial compliance. The capacitive compliance of large arteries (C1), including the aorta, and the endothelial function of small arteries (C2) were automatically assessed as a mean of the five most similar pulse waves appearing during 30-s of measurement. Three consecutive measurements were performed to obtain mean results for every participant.

Carotid-femoral PWV was measured using the foot-to-foot velocity method from carotid and femoral waveforms by employing a SphygmoCor device (AtCor Medical, Sydney, Australia). Transcutaneous readings were obtained at the right common carotid artery and the right femoral artery with the subjects in a supine position with direct-contact pulse sensors. The time delay (Dt or transit time) of the two waveforms was registered, and the distance (D) between carotid and femoral recording sites was obtained by subtracting the carotid measurement site to sternal notch distance from the sternal notch to the femoral measurement site distance. PWV was calculated as follows: D/Dt (m/s) [29, 30]. Three measurements were performed to obtain average results for every participant. Only measurements that met the automatic quality control cutoff were used in the final analysis. All the PWV measurements were performed by two experienced nurses.

**Statistical analysis**

The data were analyzed by using IBM® SPSS® Statistics Version 23 software (copyright 2015). Variables were tested for normality by way of Shapiro–Wilk or Kolmogorov–Smirnov tests, as appropriate. Data are presented as mean ± standard deviation (SD) if not mentioned otherwise. Differences in continuous variables between GDM participants and controls were studied by using Student’s t test in cases of normality and the Mann–Whitney U test in cases of skewed distribution of measurements.

All 240 women were also included in subgroup analyses to study the effect of obesity on the results. For these analyses, we divided the whole study group into four subgroups according to obesity and previous GDM. Obesity was classified as BMI ≥30 kg/m² [47]. The clinical characteristics of these four subgroups were studied by way of one-way ANOVA in cases of normality and by using the Kruskal–Wallis test in cases of non-normality. If the overall p value was significant, individual p values between subgroups were also calculated. Post hoc analyses, with a conservative Bonferroni correction factor, were performed in order to correct for multiple testing. The relationships between different cardiovascular risk factors were studied by Pearson's or Spearman's correlation analysis, as appropriate.

Further, we conducted univariate linear regression analyses for hsCRP, MMP-8, TIMP-1, PWV and arterial
compliance index values to find possible associations with clinically relevant covariates. Then multivariable linear analyses were carried out to examine whether simple associations were changed after adjustment for potential confounders. Finally, stepwise multiple linear regression analyses were done to find out relevant covariates to final models. The selected covariates in all of these analyses were age, BMI, previous GDM, time after the index pregnancy, pack-years of smoking, heart rate, systolic blood pressure, hsCRP, TC and fasting insulin. F-statistics was used to optimize the sequential variable selection procedure. A two-tailed probability value of <0.05 was considered significant.

Results
The basic clinical characteristics of the study participants are summarized in Table 1. There were no significant differences between the two cohorts in self-reported history of respiratory infection, other infectious disease or trauma during the month before follow-up laboratory examinations.

Subclinical inflammation
Serum TIMP-1 levels were significantly increased after previous GDM (Table 2). There was a significant positive association between previous GDM and TIMP-1 levels in both univariate and multivariable linear regression analyses (data not shown). There were no differences in the concentrations of MMP-8 and MMP-9 between the groups (Table 2). In stepwise multiple linear regression analyses, hsCRP, previous GDM and TC were important determinants of MMP-8 levels. Likewise, previous GDM, together with BMI and heart rate associated with TIMP-1 in stepwise multiple linear regression analyses. Nevertheless, the significant determinants explained only 13.8% of MMP-8 and 6.7% of TIMP-1 concentrations (Table 3).

We found no difference in the concentrations of hsCRP between GDM cases and controls (Table 2), even when participants affected with infections or traumas were excluded (data not shown). In stepwise multiple linear regression analysis (Table 3), only BMI was a significant determinant of hsCRP levels, but the model explained only 9.6% of hsCRP values. Previous GDM did not influence hsCRP concentrations in our data.

Pulse wave velocity and arterial compliance
PWV values differed significantly between the GDM cases and controls (Table 2). In univariate linear regression analysis, there were significant associations with age (p < 0.001), fasting insulin (p < 0.001), previous GDM (p = 0.009), TC (p < 0.001), heart rate (p < 0.001), systolic blood pressure (p < 0.001) and BMI (p < 0.001). In stepwise multiple linear regression analysis, significant determinants of PWV values were systolic BP, age, insulin levels, previous GDM and time after the index pregnancy. Covariates explained 47.0% of PWV (Table 3). In our two study cohorts, there were no interactions between previous GDM and TIMP1 on PWV (data not shown).

There was a nonsignificant difference in C1 values between the study groups. No difference was revealed in C2 values, either. In univariate linear regression analysis,

Table 2 Results of primary analyses of GDM and control groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP, mg/L</td>
<td>2.50 ± 3.69</td>
<td>2.50 ± 4.19</td>
<td>0.582</td>
</tr>
<tr>
<td>MMP-8, ng/mL</td>
<td>27.83 ± 1.48</td>
<td>32.78 ± 1.90</td>
<td>0.082</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>384.27 ± 13.15</td>
<td>392.15 ± 12.60</td>
<td>0.667</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>102.80 ± 29.72</td>
<td>94.58 ± 24.51</td>
<td>0.020</td>
</tr>
<tr>
<td>MMP-8/TIMP-1, mol ratio</td>
<td>0.13 ± 0.009</td>
<td>0.17 ± 0.015</td>
<td>0.035</td>
</tr>
<tr>
<td>MMP-9/TIMP-1, mol ratio</td>
<td>1.32 ± 0.078</td>
<td>1.43 ± 0.085</td>
<td>0.152</td>
</tr>
<tr>
<td>C1, mL/mmHg × 10</td>
<td>15.14 ± 3.51</td>
<td>15.85 ± 3.36</td>
<td>0.092</td>
</tr>
<tr>
<td>C2, mL/mmHg × 100</td>
<td>8.44 ± 3.08</td>
<td>8.60 ± 3.20</td>
<td>0.681</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>6.44 ± 0.83</td>
<td>6.17 ± 0.74</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD
hsCRP high-sensitivity C reactive protein, C1 large artery compliance index, C2 small artery compliance index, PWV pulse wave velocity, MMP-8 matrix metalloproteinase-8, MMP-9 matrix metalloproteinase-9

Table 1 Basic clinical characteristics of women with GDM and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average time since delivery, years</td>
<td>3.7 ± 1.0</td>
<td>3.7 ± 0.9</td>
<td>0.818</td>
</tr>
<tr>
<td>Age, years</td>
<td>35.8 ± 4.4</td>
<td>35.9 ± 4.6</td>
<td>0.854</td>
</tr>
<tr>
<td>Primiparous, n (%)</td>
<td>23 (19.2%)</td>
<td>23 (19.2%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Therapy of GDM during pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, n (%)</td>
<td>24 (20.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin, n (%)</td>
<td>1 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary therapy, n (%)</td>
<td>95 (79.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>3.8 ± 6.0</td>
<td>2.4 ± 4.6</td>
<td>0.012</td>
</tr>
<tr>
<td>During the previous month, history of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory infection, n (%)</td>
<td>45 (37.5%)</td>
<td>44 (36.7%)</td>
<td>0.854</td>
</tr>
<tr>
<td>Other infectious disease, n (%)</td>
<td>18 (15.0%)</td>
<td>10 (8.3%)</td>
<td>0.053</td>
</tr>
<tr>
<td>Trauma, n (%)</td>
<td>9 (7.5%)</td>
<td>5 (4.2%)</td>
<td>0.264</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3 ± 5.0</td>
<td>27.5 ± 5.4</td>
<td>0.069</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>122.4 ± 12.5</td>
<td>119.0 ± 11.5</td>
<td>0.034</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73.5 ± 9.0</td>
<td>71.8 ± 8.7</td>
<td>0.176</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>65.9 ± 9.1</td>
<td>63.8 ± 9.6</td>
<td>0.017</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.7 ± 0.9</td>
<td>4.6 ± 0.8</td>
<td>0.329</td>
</tr>
<tr>
<td>F-Gluc, mmol/L</td>
<td>5.6 ± 0.6</td>
<td>5.3 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F-Insu, μU/L</td>
<td>5.2 ± 3.6</td>
<td>4.6 ± 3.6</td>
<td>0.087</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD
BMI body mass index, BP blood pressure, F-Gluc fasting glucose, F-Insu fasting insulin, TC total cholesterol
there was no significant association between C2 and BMI ($p = 0.726$), but an inverse association between C1 and BMI was significant ($p = 0.025$). In stepwise multiple linear regression analysis, systolic BP, heart rate, BMI and time after the index pregnancy were significant covariates explaining 52.4% of C1 values. Significant determinants of C2 values were systolic BP, heart rate, BMI, age and pack-years of smoking. These covariates explained 31.7% of C2 values (Table 3).

**Effect of obesity in subgroups**

Altogether, there were 75 women in the obese group (BMI ≥ 30 kg/m²); 43 GDM and 32 control participants. The non-obese group (BMI < 30 kg/m²; n = 165) consisted of 77 GDM and 88 control participants [55]. In subgroup analyses, participants in obese subgroups had higher serum concentrations of hsCRP than those in non-obese subgroups, as shown in Fig. 1. The concentrations of MMP-8 in the four subgroups were as follows: obese GDM cases, 27.76 ± 1.77 ng/mL, obese controls 37.10 ± 4.16 ng/mL, non-obese GDM cases, 27.88 ± 2.08 ng/mL and non-obese controls, 31.21 ± 2.10 ng/mL. The concentration of MMP-8 was highest among obese controls, but the differences between the four subgroups were not significant ($p = 0.090$). We also found no differences in the levels of MMP-9 or TIMP-1 between these four subgroups (data not shown). In the four subgroups, differences in PWV values were significant, but differences in both C1 and C2 values were not (Figs. 2, 3).

**Table 3 Results of stepwise multiple linear regression analyses**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Covariates included in the model</th>
<th>$R^2$ for model</th>
<th>Global $p$</th>
<th>Standardized $β$</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP</td>
<td>BMI</td>
<td>0.096</td>
<td>&lt;0.001</td>
<td>0.259</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMP-8</td>
<td>hsCRP</td>
<td>0.138</td>
<td>&lt;0.001</td>
<td>0.312</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Previous GDM</td>
<td></td>
<td></td>
<td>−0.137</td>
<td>0.025</td>
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<tr>
<td></td>
<td>TC</td>
<td></td>
<td></td>
<td>0.129</td>
<td>0.036</td>
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<tr>
<td>TIMP-1</td>
<td>Previous GDM</td>
<td>0.067</td>
<td>0.003</td>
<td>0.157</td>
<td>0.015</td>
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<tr>
<td></td>
<td>BMI</td>
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<td></td>
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<td>0.025</td>
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<tr>
<td></td>
<td>Heart rate</td>
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<td>−0.132</td>
<td>0.044</td>
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<tr>
<td>C1</td>
<td>Systolic BP</td>
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<td>&lt;0.001</td>
<td>−0.602</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Heart rate</td>
<td></td>
<td></td>
<td>−0.347</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td></td>
<td></td>
<td>0.232</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Time after the index pregnancy</td>
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<td></td>
<td>−0.095</td>
<td>0.041</td>
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<tr>
<td>C2</td>
<td>Systolic BP</td>
<td>0.317</td>
<td>&lt;0.001</td>
<td>−0.345</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td></td>
<td></td>
<td>−0.312</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td></td>
<td></td>
<td>0.286</td>
<td>&lt;0.001</td>
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<td></td>
<td>Age</td>
<td></td>
<td></td>
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<td>0.001</td>
</tr>
<tr>
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<td>Pack-years of smoking</td>
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<td></td>
<td>−0.144</td>
<td>0.012</td>
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<td>PWV</td>
<td>Systolic BP</td>
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<td>&lt;0.001</td>
<td>0.534</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Age</td>
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<td>0.230</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>F-Insu</td>
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<td></td>
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<td>&lt;0.001</td>
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<td>Previous GDM</td>
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<td>0.026</td>
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<tr>
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<td>Time after the index pregnancy</td>
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<td></td>
<td>−0.102</td>
<td>0.040</td>
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</tbody>
</table>

Covariates in these analyses included age, BMI, previous GDM, pack-years of smoking, time after the index pregnancy, heart rate, systolic blood pressure, hsCRP, TC and fasting insulin. Final models include significant covariates only. Standardized $β$ provides a measure of the relative strength of an association, independent of the measurement units. Standardized $β$ and $p$ values are shown only when $p < 0.05$.

Discussion
Our main finding was that PWV was significantly higher after GDM than after normoglycemic pregnancy. This was supported by a nonsignificant difference in the large-artery compliance index, C1, which indicates that the arteries of GDM cases were less distensible than those of the controls. Secondly, subclinical low-grade inflammation and reduced arterial compliance especially affected women with high BMI.

Inflammation has been shown to be a strong predictor of women’s cardiovascular complications [48]. We found that levels of TIMP-1 were significantly upregulated after previous GDM, reflecting low-grade inflammation...
among this relatively healthy and young study population. No differences were found in circulating levels of MMP-8 or MMP-9 between the two study cohorts. In subgroup analyses, the highest levels of MMP-8 were in obese controls, but this did not reach statistical significance either. A search of MEDLINE (English language; 1989–September 2016; search terms: “MMP-8, MMP-9, TIMP-1” and “GDM”) revealed no publications concerning female populations where levels of MMP-8, MMP-9 or TIMP-1 have been studied in connection with previous GDM.

There is evidence that glucose can modulate the expression, production and activity of MMPs. For example, endothelial cells cultured in hyperglycemic conditions present increased expression and activity of MMP-9 [49]. It is a pity that there were no samples left for MMP analysis taken from the patients during the period when they suffered from gestational diabetes. We might postulate, that during the pregnancy GDM increase concentrations of MMPs and they in turn upregulate TIMP-1. After the delivery, the decreasing concentrations of glucose, MMPs and TIMP-1 take place consecutively. The prolonged upregulation of TIMP-1 found in this study without upregulated MMP levels may also be a result of the fact that upregulated TIMP-1 may suppress MMP-8 and MMP-9 levels. Further, third explanation for prolonged TIMP-1 upregulation found in this work may be that prolonged elevation of TIMP-1 levels may mediate MMP-independent pro-inflammatory or growth-factor-like signaling functions contributing to low-grade inflammation [36–38].

Recent studies have reported higher CRP and hsCRP levels in women with a history of GDM than in age-matched normal controls after a 1- or 5-year postpartum period [4, 50, 51]. On the contrary, Ajala et al. found no difference in CRP in women after previous GDM compared to controls 4–10 years postpartum [52]. In our study, when hsCRP was determined on average at 3.7 years after delivery, there was no difference between the age-matched study cohorts. However, low-grade inflammation was evident among obese women, in contrast to non-obese participants in subgroup analyses. The GDM and non-GDM women of our study did not differ in BMI, which can partly explain the similar hsCRP levels between the two study cohorts.

Only a few studies have been published concerning a possible relationship between PWV and previous GDM. Lekva et al. reported an enhanced cardiovascular risk at 5-year follow-up as reflected in elevated PWV after previous GDM diagnosed using the old criteria of the World Health Organization (WHO) (OGTT: 2-h plasma glucose ≥7.8 mmol/L). However, they did not find such an association in PWV when using IADPSG diagnostic criteria (OGTT: fasting plasma glucose 5.1–6.9 mmol/L, 1-h plasma glucose ≥10.0 mmol/L or 2-h plasma glucose 8.5–11.0 mmol/L) [41, 53]. Using diagnostic criteria of GDM similar to those of the IADPSG [39], we observed a significant increase in PWV in women with previous GDM. Previous GDM was also a significant determinant of PWV in multiple linear regression analysis. Our results are in accordance with those of Tam et al., who reported higher PWV in women with a history of GDM followed up at a median of 6 years postpartum [54]. In contrast to these findings, Heitritter et al. detected no difference in PWV at an average of 1 year after previous GDM compared with normoglycemic pregnancy [4]. There were no significant differences in C1 or C2 values between the GDM cases and controls. In a recent study, no difference was found in vascular function measured also by using HDI/PulseWave™CR-2000 in women with a history of GDM when compared to healthy controls 4–10 years postpartum, either [52].

Strengths of our study include the fact that we used standardized measurements of arterial stiffness. Determination of systemic arterial stiffness by using HDI/PulseWave™CR-2000 equipment is widely used, and carotid-femoral PWV is accepted as the most reliable measurement of arterial stiffness [29]. We measured the levels of MMP-8, MMP-9 and TIMP-1 by specific immunoassays previously found to be suitable for diagnosis and monitoring of systemic low-grade inflammation associated with cardiovascular and infectious diseases as well as other inflammatory states [11, 13–18, 23–25]. Further, we performed a well characterized hospital-based study of two cohorts of women with a similar follow-up time and age. Moreover, there was no significant difference in BMI between the study groups, and all participants had undergone OGTT screening during the index pregnancy. Since low-risk parturients do not routinely undergo OGTTs in Finland [39], this last strength may also turn out to be a weakness, because the most low-risk women had to be excluded from our study [40]. Although the relatively short time from delivery to the follow-up study allowed us to observe early cardiovascular changes, it may be one of our study limitations as well, since major differences between the study groups are probably better observable later in their life. For example, within 7 years postpartum, previous GDM was identified as a risk factor of CVD by Goueslard et al. They studied database of more than 1.5 million deliveries and found that the incidence of myocardial infarction was 0.04% in women with a history of GDM and 0.02% without [7].

In our subgroup analyses, obesity was associated with higher levels of hsCRP and higher values of PWV. We have earlier revealed the effect of obesity being similar with many other markers for cardio-metabolic risks
among the four subgroups [40, 55]. Earlier, BMI has been shown to associate inversely with arterial compliance [56]. As presented in Fig. 3, this seemed to be the case also in our study in C1 values. Surprisingly, in multiple regression analyses, BMI seemed to be protective as regards arterial compliance (C1 and C2). BMI was significantly correlated with systolic blood pressure and heart rate (data not shown). Hence, adjusted findings concerning C1 and C2 might have been affected by these relationships irrespective of possible biologic associations. In our opinion, this result may be explained by multiple interactions of C1 and C2 measurements with other confounding variables. This was supported by the findings of univariate analysis and stepwise multiple linear regression analysis without systolic BP and heart rate as covariates, where inverse association between BMI and C1 was found and association between BMI and C2 was vanished (data not shown).

The prevalence of obesity is increasing around the world [57]. Specifically, visceral obesity modifies glucose and lipid metabolism. It is associated with increased risk of arterial stiffness and atherosclerosis both in normal-weight subjects and patients with T2DM [58, 59]. Our results imply that in preventing cardiovascular risk among women after delivery, we need a comprehensive attitude in clinical care instead of concentrating on single factors.

Conclusions
When studied 3.7 years after delivery, PWV values were higher in women with previous GDM, indicating that their arteries are less distensible than those in women with previous normoglycemic pregnancy. Among other findings, this relationship was even more evident in obese subjects. We also found that serum levels of TIMP-1 were significantly upregulated after previous GDM, reflecting low-grade inflammation among this relatively healthy and young study population. Altogether, our results demonstrate that previous GDM may reflect a subclinical inflammatory state and together with obesity may contribute to an early stage of the subclinical atherosclerotic process even in relatively young and healthy women.

Abbreviations
C1: large artery compliance index; C2: small artery compliance index; GDM: gestational diabetes mellitus; hsCRP: high-sensitivity C-reactive protein; MMP: matrix metalloproteinase; OGTT: oral glucose tolerance test; PWV: pulse wave velocity; TIMP: tissue inhibitor of metalloproteinase.

Authors' contributions
TV-K participated in the design of the study, conducted experiments, performed data analyses and drafted the manuscript. AL participated in the design of the study and contributed to drafting the manuscript. TT carried out the analyses of MMP-8, MMP-9 and TIMP-1. OP, IU and TS contributed to drafting the manuscript. AP designed the study, helped to perform data analyses and drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets generated and analyzed during the current study are not publicly available as a result of the fact that individual privacy could be compromised, but are available from the corresponding author on reasonable request.

Consent for publication
Every participant was given both oral and written information on the study before she signed an informed consent document.

Ethics approval and consent to participate
The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Kanta-Häme Hospital District (Reference Number 521/2010; date of approval 21.12.2010).

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References


Arterial stiffness in fertile women with metabolic syndrome

Running title: Arterial stiffness in women with MetS

Original Article

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Abstract

Introduction: Although metabolic syndrome (MetS) is evidently associated with the risk of cardiovascular disease (CVD), recently its use has been questioned. We studied the utility of MetS diagnosis when estimating individual CVD risk.

Methods: We compared 27 fertile women with MetS and 27 counterparts without the syndrome, matched pairwise according to well-known risk factors of CVD. Pulse wave velocity (PWV) and central blood pressure (cBP) were determined noninvasively via a SphygmoCor device. Arterial compliance was measured noninvasively with an HDI/PulseWave™CR-2000 arterial tonometer.

Results: PWV (7.1 ± 2.5 vs. 6.5 ± 1.1 m/s, P = 0.037), and both systolic (120.9 ± 12.2 vs. 111.5 ± 16.0 mmHg, P = 0.031) and diastolic cBP (81.3 ± 8.5 vs. 74.1 ± 11.2 mmHg, P = 0.035) were higher in the MetS group. Systemic arterial compliance values were lower in both large (15.1 ± 8.0 vs. 16.1 ± 4.4 mL/mmHg×10, P = 0.034) and small arteries (7.1 ± 2.5 vs. 9.3 ± 3.2 mL/mmHg×100, P = 0.010) in women with MetS.

Conclusions: Fertile women with MetS had increased arterial stiffness, as measured by three different methods. Our results highlight the utility of MetS when revealing increased individual CVD risks in fertile-aged women.

Keywords: arterial compliance, arterial stiffness, cardiovascular disease, central blood pressure, gestational diabetes mellitus, metabolic syndrome, pulse wave velocity
Key messages:

- Women with MetS have increased arterial stiffness when measured by different methods.

- MetS is a useful clinical tool to assess increased cardiovascular risk, particularly among fertile-aged women.
Introduction

Metabolic syndrome (MetS) is defined as a group of risk factors related to increased risks of cardiovascular diseases and diabetes (1). Although many diagnostic criteria have been proposed for MetS since the 1980s, hyperglycemia, dyslipidemia, hypertension, and abdominal obesity are recognized as key components (2). In recent decades the prevalence of MetS has increased significantly in parallel with the global epidemic of obesity (3). Although the presence of MetS is associated with an increased risk of CVD (1,4,5), the results of the large INTERHEART study suggested that the use of dichotomous risk factors used in MetS classification may underestimate future CVD risk (6).

Cardiovascular diseases (CVDs) are the leading causes of female mortality, responsible for one third of deaths in women globally (7,8). The appearance of CVD can differ between the sexes, making the identification of CVD in women challenging (9,10). Pregnancy can reveal a woman´s tendency to be at an increased risk of health problems later in life. Growing evidence suggests that women with a history of gestational diabetes mellitus (GDM) are at an increased risk of CVD, type 2 diabetes or MetS later in life (11-14).

Arterial stiffness is an important marker of arteriosclerosis, predicting future CVD events (15-18). With aging, the wall of the artery loses elasticity and becomes rigid (19-21). Measurement of carotid to femoral pulse wave velocity (PWV) as a marker of aortic stiffness has emerged as the gold standard method (18). There are also other ways to measure arterial stiffness noninvasively. Systemic arterial compliance can be determined by using radial artery pulse wave analysis (18,22). Central blood pressure (cBP) registered noninvasively seems to be more relevant than peripheral BP as regards the pathogenesis of CVD (23,24). It also correlates with cardiovascular risk in healthy people (25).
Weighing the possible value of MetS may be related to individual perspectives, i.e. the point of view of an epidemiologist may be different from that of a clinical physician. Hence, the value of assessing MetS per se when estimating individual cardiovascular risk has been questioned (6,26-29). We aimed to study this by pairwise matching of fertile-aged women with and without MetS, in relation to well-known risk factors of CVD. Our special interest was to determine whether or not there are differences in pulse wave velocity, central blood pressure and systemic arterial compliance between fertile-aged women with and without MetS.

**Material and methods**

**Study population**

This cross-sectional study was performed at Kanta-Häme Central Hospital and Linnan Klinikka, Hämeenlinna, Finland. The complete study protocol has been described in detail previously (14). In brief, we investigated a total of 120 parturients from our area with a history of GDM during the index pregnancy and we compared them with 120 age-matched women with normal glucose metabolism during pregnancy. Index pregnancies and deliveries were 2–6 years before participating in the study. GDM was defined as a pathological value in a 75-g oral glucose tolerance test (OGTT) during pregnancy: venous plasma glucose ≥ 5.3 mmol/L when fasting, ≥ 10.0 mmol/L at 1 hour or ≥ 8.6 mmol/L at 2 hours. The diagnostic criteria of GDM were the same as in current Finnish guidelines (30). MetS was defined according to the National Cholesterol Education Program (NCEP Adult Treatment Panel III), and for women this is the presence of at least three of the following five criteria (2): waist circumference > 88 cm; serum triglycerides ≥ 1.7 mmol/L; serum high-density lipoprotein cholesterol (HDL-C) level < 1.3 mmol/L; blood pressure ≥ 130/85 mmHg; plasma glucose level ≥ 6.1 mmol/L or diabetes mellitus.
We found 2.4-fold increased risk of MetS after previous GDM when compared with normoglycemic pregnancies (14). In the current analysis, we included all 27 women with MetS from a total of 240 participants in our original study. Every woman with MetS was compared with an individually paired counterpart without MetS. To avoid the confounding effects of well-known cardiovascular risk factors, the counterparts without MetS were matched according to age, previous GDM status, and serum concentrations of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) (Table 1). All the participants were of Caucasian origin. Both recruitment and examinations were carried out between January 2013 and July 2014.

Table 1. Parameters matched among MetS participants and their counterparts without MetS.

<table>
<thead>
<tr>
<th>Matching parameter</th>
<th>MetS (n = 27)</th>
<th>Control (n = 27)</th>
<th>P value</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>36.8</td>
<td>4.7</td>
<td>36.6</td>
<td>4.5</td>
<td>0.2</td>
<td>-2.3</td>
<td>2.7</td>
<td>0.880</td>
<td></td>
</tr>
<tr>
<td>Previous GDM, n (%)</td>
<td>19</td>
<td>70</td>
<td>19</td>
<td>70</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.1</td>
<td>1.2</td>
<td>5.2</td>
<td>0.9</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.4</td>
<td>0.9</td>
<td>3.3</td>
<td>0.8</td>
<td>0.1</td>
<td>-0.4</td>
<td>0.5</td>
<td>0.768</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; GDM: gestational diabetes mellitus; LDL-C: low-density lipoprotein cholesterol; MetS: metabolic syndrome; TC: total cholesterol

We interviewed the participants as regards their medical histories and lifestyle habits. To analyze “yo-yo” dieting, we estimated total lifetime weight loss by adding together the kilograms lost during every previous intentional weight-loss period. Lifetime tobacco exposure was calculated as pack-years by multiplying years of smoking by the average
number of packs smoked daily (31). One pack-year is defined as twenty cigarettes smoked every day for one year.

Resting heart rate and brachial blood pressure of the participants was assessed automatically by using CR-2000 equipment (HDI/PulseWave™CR-2000, Hypertension Diagnostics, Inc., Eagan, Minnesota, USA) during the measurement of arterial compliance. The mean of three measurements was used in the analysis. Weight (kg), height (cm) and waist circumference (cm) were measured according to general recommendations. Waist circumference (WC) was measured midway between the lowest rib and the iliac crest. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²).

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (32), and the protocol was approved by the Ethics Committee of Kanta-Häme Hospital District (reference number 521/2010; date of approval 21.12.2010). Every participant was given both oral and written information on the study before she signed an informed consent document.

Laboratory Methods

Basic blood count and serum levels of creatinine, alanine transaminase (ALAT), fasting glucose and insulin, glycosylated hemoglobin (HbA1c), TC, HDL cholesterol, LDL cholesterol and triglycerides, and the urinary albumin to creatinine ratio, were analyzed according to validated methods as described in detail earlier after at least 12 hours of fasting (14,33). Serum concentrations of high-sensitivity C-reactive protein (hsCRP) were analyzed according to validated immunonephelometric (United Medix Laboratories Ltd., Espoo, Finland) and immunoturbidimetric (VITA Healthcare Services Ltd., Vita Laboratory, Helsinki, Finland) methods (34,35). Plasma concentrations of oxidized low-density lipoprotein (oxLDL) were determined by using a validated ELISA method (Mercodia AB, Uppsala,
Sweden). The assay kits include the same monoclonal antibody (4E6) as originally described by Holvoet et al. (36,37).

The homeostasis model assessment of insulin resistance (HOMA-IR) index is based on measurement of plasma glucose and insulin in a single sample and is commonly used as a parameter of the severity of insulin resistance (38). It was calculated in the following way: fasting insulin (mU/L) × fasting blood glucose (mmol/L)/22.5 (39).

**Determination of arterial stiffness and compliance**

Carotid–femoral PWV was measured by using the foot-to-foot velocity method from carotid and femoral waveforms, using a SphygmoCor device (AtCor Medical, Sydney, Australia). These were obtained transcutaneously at the right common carotid artery and the right femoral artery, with the subject in a supine position, with direct-contact pulse sensors. The time delay (Dt or transit time) of the two waveforms was registered, and the distance (D) between the carotid and femoral recording sites was obtained by subtracting the distance between the carotid measurement site to the sternal notch from the distance between the sternal notch and the femoral measurement site. PWV was calculated as follows: D/Dt (m/s) (18,25). PWV increases in stiff or less distensible arteries (23,25). Three measurements were performed to obtain average results for every participant. Only measurements that met the automatic quality-control cutoff were used in the final analysis.

Central BP was estimated non-invasively from a radial artery pulse wave (SphygmoCor device; AtCor Medical, Sydney, Australia), which involves use of a radial pulse and a validated generalized transfer function to estimate central pressures from brachial BP and peripheral pulse waves (25). Three consecutive measurements were performed to obtain mean results for every participant. Values of cBP are indirect surrogate measures of arterial stiffness, but they provide additional information concerning pulse wave reflections (18).
Radial artery pulse waves were measured non-invasively with an arterial tonometer (HDI/PulseWave\textsuperscript{TM} CR-2000, Hypertension Diagnostics, Inc., Eagan, Minnesota, USA), which involves use of a modified Windkessel pulse-contour method (40). This technique is based on an assumed model of the circulation which identifies reflections in diastole as a decaying sinusoidal wave (18,41). The equipment automatically records the proximal capacitive compliance of large arteries (C1), including the aorta, and the distal oscillatory compliance, which concerns endothelial function of the microvascular circulation or small arteries (C2) (18,41). During thirty seconds of measurement, values of C1 and C2 were automatically assessed as the mean of the five most similar pulse waves appearing. Three measurements were performed to obtain mean values for every participant. Arterial compliance describes the ability of an artery to expand as a response to pulse pressure. Compliance can be understood as the inverse of stiffness – in a stiff artery compliance is low (42).

Recordings of PWV, cBP, C1 and C2 were carried out in the morning after at least ten minutes of rest in a semi-sitting position. The participants were asked to refrain from eating, drinking caffeinated drinks, smoking and taking medication for 12 hours, and drinking alcohol for two days prior to measurement. All the measurements were performed by four experienced nurses.

**Statistical analysis**

Statistical analysis was carried out by using IBM\textsuperscript{®} SPSS\textsuperscript{®} Statistics Version 23 software (copyright 2015). Variables were tested for normality by way of Shapiro–Wilk tests. Data are presented as mean ± standard deviation (SD) if not mentioned otherwise. Differences in continuous variables between MetS participants and paired counterparts were studied by using paired \( t \) test in cases of normality and by the Wilcoxon test in cases of non-normality. Differences in binomial outcomes between the two paired study groups were tested by using McNemar’s test. The Hodges-Lehmann estimate was used for calculating the difference
between MetS and their matched controls medians and 95% confidence interval (CI) for the difference. A two-tailed probability value of < 0.05 was considered significant.

**Results**

Variables of MetS defined according to NCEP Adult Treatment Panel III for women with MetS and their matched counterparts without MetS are shown in Table 2. There were no differences in family history of coronary heart disease, cerebrovascular disease or diabetes mellitus between the study groups (data not shown). In individual pairwise comparisons, no differences were found in diagnosed disorders or permanent medication for any chronic disease (data not shown). Further, there was no difference in current smoking in individual pairwise comparisons (6 vs. 4, \(P = 0.728\)).

**Table 2.** Components of MetS in the MetS women and their matched controls without the syndrome.

<table>
<thead>
<tr>
<th>Determinant of MetS</th>
<th>MetS (n = 27)</th>
<th>Control (n = 27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>107.7</td>
<td>11.0</td>
<td>97.8</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>135.7</td>
<td>13.6</td>
<td>125.9</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>78.4</td>
<td>8.1</td>
<td>73.0</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.7</td>
<td>0.6</td>
<td>5.4</td>
</tr>
<tr>
<td>T2DM, n (%)</td>
<td>1*</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.7</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.2</td>
<td>0.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

BP: blood pressure; CI: confidence interval; GDM: gestational diabetes mellitus; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; T2DM: type 2 diabetes mellitus

* T2DM in a woman with previous GDM
Baseline characteristics and laboratory findings in both groups are shown in Table 3. Body mass index was higher in the women with MetS, but their paired counterparts were also overweight (Table 3). Heart rate was 67.9 (± 8.8) beats per minute (bpm) in the MetS group and 65.7 (± 10.6) bpm among the paired controls (Difference = 2.2; 95% CI: -2.2, 6.6; P = 0.211). There were no differences in the concentrations of white blood cells or platelets between the groups (data not shown), but that of hemoglobin was higher among women with MetS (Table 3). The concentration of HbA1c was 34.6 (± 2.9) mU/L in the MetS group, and 34.7 (± 2.5) mU/L in the paired controls (Difference = -0.1; 95% CI: -1.7, 1.4; P = 1.000). The urinary albumin to creatinine ratio was significantly higher among women with MetS, 0.7 (± 0.4) mg/mmol vs. 0.5 (± 0.3) mg/mmol, Difference = 0.2; 95% CI: 0.0, 0.4 (P = 0.034), respectively.
**Table 3.** Baseline characteristics and laboratory findings in the MetS women and their matched controls without the syndrome.

<table>
<thead>
<tr>
<th></th>
<th>MetS</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 27</td>
<td>n = 27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Difference 95% CI</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>4.1 8.7</td>
<td>1.9 4.8</td>
<td>2.1 -1.8, 6.0 0.276</td>
</tr>
<tr>
<td>Alcohol intake, g/day</td>
<td>1.1 1.4</td>
<td>1.5 1.6</td>
<td>-0.6 -1.4, 0.1 0.242</td>
</tr>
<tr>
<td>Lifetime weight loss, kg</td>
<td>30.4 31.4</td>
<td>28.0 35.2</td>
<td>2.4 -18.7, 23.6 0.657</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.5 6.2</td>
<td>28.9 5.0</td>
<td>4.6 1.2, 7.9 0.010</td>
</tr>
</tbody>
</table>

**Clinical chemistry**

<table>
<thead>
<tr>
<th></th>
<th>MetS</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Difference 95% CI</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>138.2 6.9</td>
<td>130.5 9.1</td>
<td>7.2 2.5, 11.9 0.004</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>3.6 4.1</td>
<td>3.7 5.2</td>
<td>-0.1 -2.7, 2.6 0.516</td>
</tr>
<tr>
<td>oxLDL, U/L</td>
<td>48.3 14.6</td>
<td>48.0 17.1</td>
<td>0.3 -8.1, 8.7 0.942</td>
</tr>
<tr>
<td>F-insu, mU/L</td>
<td>9.0 5.9</td>
<td>6.4 4.3</td>
<td>2.6 -0.5, 5.7 0.073</td>
</tr>
<tr>
<td>ALAT, U/L</td>
<td>32.3 24.1</td>
<td>22.2 20.5</td>
<td>10.3 0.6, 19.5 0.022</td>
</tr>
<tr>
<td>Crea, µmol/L</td>
<td>65.3 9.0</td>
<td>64.6 5.4</td>
<td>0.7 -3.8, 5.2 0.748</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.3 1.5</td>
<td>1.6 1.1</td>
<td>0.7 -0.1, 1.5 0.046</td>
</tr>
</tbody>
</table>

ALAT: alanine transaminase; BMI: body mass index; CI: confidence interval; Crea: creatinine; F-insu: fasting insulin; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; MetS: metabolic syndrome; oxLDL: oxidized low-density lipoprotein (plasma concentration)
As measured by three different methods, arterial stiffness values differed significantly between the fertile women with MetS and their matched counterparts without the syndrome. Arterial stiffness was higher among the women with MetS than in their matched counterparts when measured by means of PWV (Figure 1), as were both systolic and diastolic cBP (Figure 2). Values of systemic arterial compliance (both C1 and C2) were significantly lower in the MetS group (Figure 3).

**Figure 1** PWV in the MetS women and their matched controls without the syndrome.

Median (minimum, maximum) PWV among matched control women was 6.3 (5.1, 9.7) m/s, and among women with MetS, 6.9 (5.9, 9.2) m/s (Difference = -0.7; 95% CI: -1.1, -0.0; P = 0.037).
Figure 2 Central systolic (A) and diastolic pressures (B) in the MetS women and their matched controls without the syndrome.

A: Median (minimum, maximum) central systolic pressure among matched control women was 107 (90, 154) mmHg and among women with MetS, 120 (97, 147) mmHg (Difference = -12.5; 95% CI: -20.3, -1.2; P = 0.031).

B: Median (minimum, maximum) central diastolic pressure among matched control women was 73 (56, 106) mmHg and among women with MetS, 81 (65, 94) mmHg (Difference = -9.3; 95% CI: -15.3, -0.7; P = 0.035).
Figure 3 Large- (A) and small-artery (B) compliance index values in the MetS women and their matched controls without the syndrome.

A: The median (minimum, maximum) large-artery compliance index value among matched control women was 15.5 (7.2, 25.7) mL/mmHg×10 and among women with MetS, 13.8 (8.8, 53.3) mL/mmHg×10 (Difference = 2.0; 95% CI: 0.4, 4.2; P = 0.034).

B: The median (minimum, maximum) small-artery compliance index value among matched control women was 9.4 (3.5, 15.3) mL/mmHg×100 and among women with MetS, 7.8 (1.8, 9.7) mL/mmHg×100 (Difference = 2.2; 95% CI: 0.7, 3.5; P = 0.010).
Discussion

Women with MetS had higher PWV values when compared with paired women without the syndrome, suggesting that MetS in fertile-aged women is associated with increased arterial stiffness. Further, women with MetS had increased cBP, as well as decreased C1 and C2 values when compared with their counterparts without MetS, thus providing further support for the finding.

Increased PWV, as a measure of arterial stiffening, is a strong predictor of cardiovascular events and mortality (43). As reviewed by Vlachopoulos et al., an increase in PWV of 1 m/s is related to a 14–15% increase in cardiovascular events, cardiovascular mortality and all-cause mortality (43). There are several plausible reasons for the current finding of increased PWV in women with MetS. Small dense LDL (sdLDL), i.e. poor quality of LDL, known to be associated with MetS and hypertriglyceridemia has found to be an important predictor of atherosclerosis (44,45). Like sdLDL, also circulating triglyceride rich lipoproteins may induce endothelial dysfunction (46,47). Chronic hyperglycemia and hyperinsulinemia promote the development of arterial wall hypertrophy by increasing local activity of the renin-angiotensin-aldosterone system (48). Moreover, high blood pressure stimulates excessive collagen production in the arterial wall (48) and insulin resistance promotes the formation of advanced glycation end-products and collagen cross-linking (49). Furthermore, decreased vasodilatory effects of insulin and free fatty acids cause impaired endothelial function (48). MetS can also be considered to be a pro-inflammatory state, which could cause endothelial dysfunction (50). All these changes in arterial wall structure and function have adverse effects on the cushioning capabilities of arteries, thus increasing arterial stiffness.

Carotid–femoral PWV is widely studied and considered as a gold standard in the evaluation of arterial stiffness (17). Arterial stiffness can also be determined by measuring cBP (17) or
compliance of large (C1) and small (C2) arteries (40). As discussed in a consensus document by Agabiti-Rosei et al. (25), increased cBP has been shown to correlate with cardiovascular risk in apparently healthy subjects and in patients with atherosclerotic disease. Moreover, decreased values of C1 and C2 have been found to be associated with MetS (51) and increased cardiovascular risk as estimated by using FINRISK and SCORE risk models (52).

We found higher cBP, and lower C1 and C2 values among fertile-aged women with MetS when compared with women without the syndrome. This provides further evidence of the negative effects of MetS on arterial stiffness among fertile-aged women. Between the study groups there was a small but significant difference in microalbuminuria. As a marker of endothelial dysfunction, this finding also highlights the effect of MetS on arterial stiffness.

The number of subjects was relatively small, but the number of patients was big enough to show the statistically significant difference between the matched groups. Hence, the confounding factors were used as matching criteria. In this setting, according to all methods used women with MetS had increased arterial stiffness.

Physical activity is known to be crucial in the prevention of CVD. Two recent studies are part of a continuum concerning research into atherosclerotic risk factors among men with MetS and physically active (PhA) men (53,54). Pohjantähti-Maaros et al. found that PhA men had better C1 values compared with MetS participants, but no difference was found as regards C2 (54). Higher numbers of smokers and greater alcohol intake were more often present among men with MetS compared with PhA subjects (54). Our study has expanded research into MetS in women. In contrast to earlier findings, there were no significant differences in pack-years of smoking or alcohol intake between the paired study groups. The apparent discrepancy of these results may be attributed to variability in selection of controls. In agreement with this, MetS per se seems to be an independent predictor of increased arterial stiffness in the present study.
Initially successful weight losses followed by weight regain (weight cycling or so called “yo-yo” dieting) is associated with body-weight excess and abdominal fat accumulation (55). Nonalcoholic fatty liver disease is commonly associated with obesity, insulin resistance, dyslipidemia and type 2 diabetes, and can thus be regarded as the hepatic manifestation of metabolic syndrome (56). We found no difference in lifetime weight loss between the paired study groups. The women in both groups were overweight. In contrast, both BMI and serum concentrations of ALAT were higher among women with MetS compared with women without the syndrome, reflecting the hepatic manifestation of MetS.

Diagnosis of MetS has been the subject of severe criticism, and it has even been suggested that MetS “should rest in peace” (57,58). The major concerns are the uncertain pathophysiology of the syndrome, the use of discrete thresholds to define abnormalities, the existence of different definitions, the exclusion of other important cardiovascular risk factors (e.g. age, sex, family history, LDL-cholesterol), and the lack of specific treatment for the syndrome (57,58). However, MetS has previously been shown to be associated with an increased risk of CVD (1,3,4,59), and the risk of CVD associated with MetS is even greater than the risk associated with the individual components (5). Moreover, it has been suggested that MetS could be a valuable public-health tool as it can be used to identify high-risk individuals at a young age (60). Our results, showing increased arterial stiffness in fertile-aged women with MetS support the use of MetS in the evaluation of CVD risk.

In conclusion, fertile-aged women with MetS have increased arterial stiffness as measured by three different methods, even when their counterpart are matched according to many other well-known CVD risk factors. The present results strongly support the clinical use of MetS as a tool for cardiovascular risk assessment, particularly among fertile-aged women.
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Disclosure of interest

The authors report no conflicts of interest.

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