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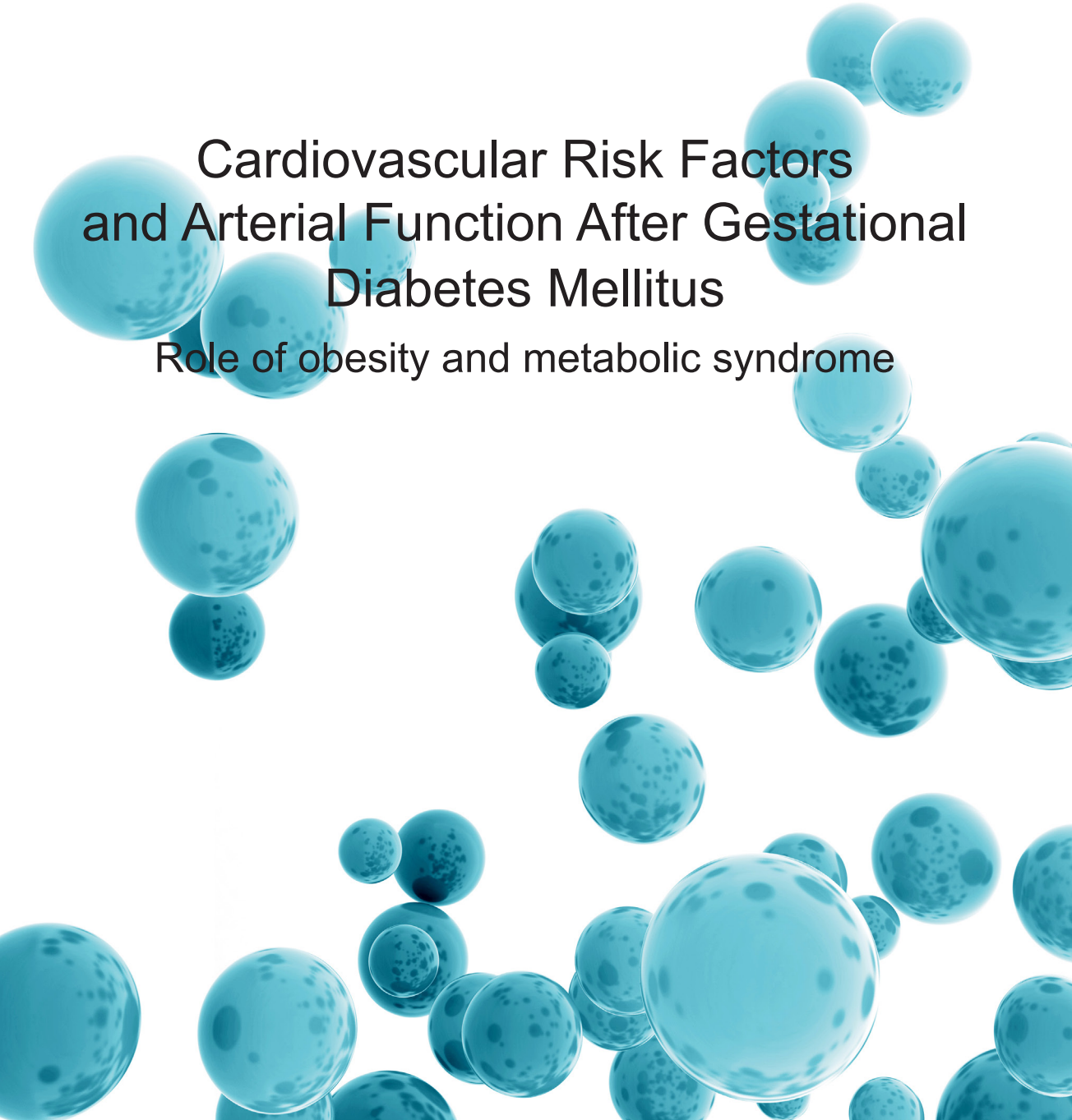
Cardiovascular Risk Factors and Arterial Function After Gestational Diabetes Mellitus

Role of obesity and metabolic syndrome



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ACADEMIC DISSERTATION
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UNIVERSITY OF TAMPERE

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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.

TIIVISTELMÄ

Raskausdiabetes eli gestationaalinen diabetes mellitus (GDM) tarkoittaa poikkeavaa glukoosiaineenvaihduntaa, joka todetaan ensimmäisen kerran raskauden aikana. Vuonna 2016 GDM komplisoi 17,5% raskauksista Suomessa. Yleensä poikkeava glukoosiaineenvaihdunta normalisoituu synnytyksen jälkeen, mutta raskausdiabeetikoilla on todettu seitsemän kertaa suurempi riski sairastua tyypin 2 diabetekseen (T2DM) myöhemmin elämänsä aikana. Lisäksi raskausdiabeetikoilla on tulevaisuudessa lisääntynyt sydän- ja verisuonitauti- sekä metabolisen oireyhtymän (MBO) riski. Jälkimmäisellä tarkoitetaan valtimotaudin riskitekijöiden kasaumaa. Vaikka MBO on liitetty kohonneeseen sydän- ja verisuonitautiriskiin, sen käyttöä kliinisessä työssä on myös kyseenalaistettu.

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

Tutkimuksen kahteen, GDM- ja kontrollikohorttiin valittiin yhteensä 240 vuosina 2008–2011 Kanta-Hämeen keskussairaalassa synnyttänyttä naista, joista 120 oli raskausaikana glukoosirasituskokeella diagnosoitu GDM ja 120 todettu normaali sokeriaineenvaihdunta. Osatöissä I–III verrattiin näiden tutkimuskohorttien seurantatutkimusten – haastattelun, fysikaalisten mittausten, laboratorio- ja valtimoiden toimintakokeiden – tuloksia MBO:n esiintyvyyden, sokeri- ja rasva-aineenvaihdunnan, matala-asteisen tulehdustilan sekä valtimoiden elastisuuden suhteen. Arvioitaessa lihavuuden vaikutusta tuloksiin tutkimuspotilaat jaettiin neljään alaryhmään GDM-statuksen sekä painoindeksin mukaan. Yhteensä 27 naisella alkuperäisestä 240 tutkimuspotilaan populaatiosta todettiin MBO. Osatyössä IV verrattiin pareittain näiden 27 MBO:ää sairastavan naisen valtimoiden elastisuustuloksia 27 tunnettujen sydän- ja verisuonitaudin riskitekijöiden suhteen täsmätyn oireyhtymää sairastamattoman naisen vastaaviin tuloksiin.

Osatöissä I–III keskimäärin 3,7 vuotta synnytyksen jälkeen tehdyissä seurantatutkimuksissa todettiin, että raskausdiabeetikoilla esiintyi 2,4-kertaisesti

metabolista oireyhtymää verrattuna raskausaikana glukoosiaineenvaihdunnaltaan terveiksi todettuihin naisiin. Myös insuliiniresistenssi oli merkittävästi yleisempää raskausdiabeteksen sairastaneilla naisilla. Matala-asteiseen tulehdusreaktioon viittaava seerumin metalloproteinaasin inhibiittoripitoisuus oli koholla raskausdiabeteksen jälkeen. Lisäksi GDM-ryhmässä naisilla oli suurempi pulssiaallon kulkunopeus viitaten kontrolliryhmän naisia jäykempiin valtimoihin. Suurin osa löydöksistä korostui lihavilla naisilla ylittäen aiemmin sairastetun GDM:n aiheuttaman vaikutuksen. Osatyössä IV tutkittiin metabolista oireyhtymää sairastavien naisten verisuonten elastisuutta. Tuloksia verrattiin tarkasti tunnettujen sydän- ja verisuonitautien riskitekijöiden suhteen täsmättyjen, mutta oireyhtymää sairastamattomien naisten tuloksiin. Kolmella ei-kajoavalla menetelmällä mitattuna metabolista oireyhtymää sairastavilla naisilla oli jäykemmät valtimot oireyhtymää sairastamattomien naisten tuloksiin verrattuna. Tulokset tukevat MBO-diagnoosin kliinistä käyttökelpoisuutta etenkin fertiili-ikäisillä naisilla.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I–IV).

I Vilmi-Kerälä T, Palomäki O, Vainio M, Uotila J, Palomäki A. The risk of metabolic syndrome after gestational diabetes mellitus – a hospital-based cohort study. Diabetology & Metabolic Syndrome 2015; 7: 43.

II Vilmi-Kerälä T, Palomäki O, Kankkunen P, Juurinen L, Uotila J, Palomäki A. Oxidized LDL, insulin resistance and central blood pressure after gestational diabetes mellitus. Acta Obstet Gynecol Scand. 2016; 95(12): 1425-1432.

III Vilmi-Kerälä T, Lauhio A, Tervahartiala T, Palomäki O, Uotila J, Sorsa S, Palomäki A. Subclinical inflammation associated with prolonged TIMP-1 upregulation and arterial stiffness after gestational diabetes mellitus: a hospital-based cohort study. Cardiovasc Diabetol. 2017; 16(1): 49.

IV Vilmi-Kerälä T, Koivistoinen T, Palomäki O, Uotila J, Palomäki A. Arterial stiffness in fertile women with metabolic syndrome. Ann Med. 2017; 49(8): 636-643.

ABBREVIATIONS

ACOG	American Congress of Obstetricians and Gynecologists
ADA	American Diabetes Association
ALAT	alanine transaminase
AlbCre	albumin to creatinine ratio
AMI	acute myocardial infarction
ANOVA	analysis of variance
BMI	body mass index
BP	blood pressure
C1	large arterial compliance
C2	small arterial compliance
C&C	Carpenter and Coustan
cBP	central blood pressure
CDA	Canadian Diabetes Association
CI	confidence interval
CV	cardiovascular
CVD	cardiovascular disease
D	distance
DM	diabetes mellitus
Dt	time delay/transit time
f	fasting
fP	fasting plasma
g	gram(s)

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

TABLE OF CONTENTS

1 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 cut-off 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 diagnostic test for diabetes and glucose intolerance (NDDG. 1979, WHO. 1999).

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 OGTTs. 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 OGTT 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/m^2 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/m^2 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/m^2 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 OGTT 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.

2015, Carpenter & Coustan. 1982, Gestational diabetes. Current Care Guidelines. 2013, Rani & Begum. 2016).

Table 1. Commonly used guidelines globally for the diagnosis of GDM.

Organization	Year	Advice for screening	Method of screening (positive cut-off)	Glucose load, g	Glucose thresholds (mmol/L)				No. of OGTT values for diagnosis
					fasting	1-h	2-h	3-h	
ACOG	2013	all except low risk	50 g GCT (≥ 7.8)	100	5.3	10.0	8.6	7.8	≥ 2
C&C	1982	none	OGTT	100	5.3	10.0	8.6	7.8	≥ 2
CDA	2013	not specified	50 g GCT (≥ 7.8)	75	5.3	10.6	8.9	—	≥ 1
EASD	1991	not specified	not specified	75	5.5 or 6.0	—	9.0	—	≥ 1
Finnish Guidelines	2013	all except low risk	OGTT	75	5.3	10.0	8.6	—	≥ 1
IADPSG	2010	universal	OGTT	75	5.1	10.0	8.5	—	≥ 1
NDDG	1979	none	50 g GCT (≥ 7.8)	100	5.8	10.5	9.2	8.0	≥ 2
NICE	2015	clinical risk	OGTT	75	5.6	—	7.8	—	≥ 1
WHO	1999	not specified	OGTT	75	7.0	—	7.8	—	≥ 1
WHO	2013	universal	OGTT	75	5.1	10.0	8.5	—	≥ 1

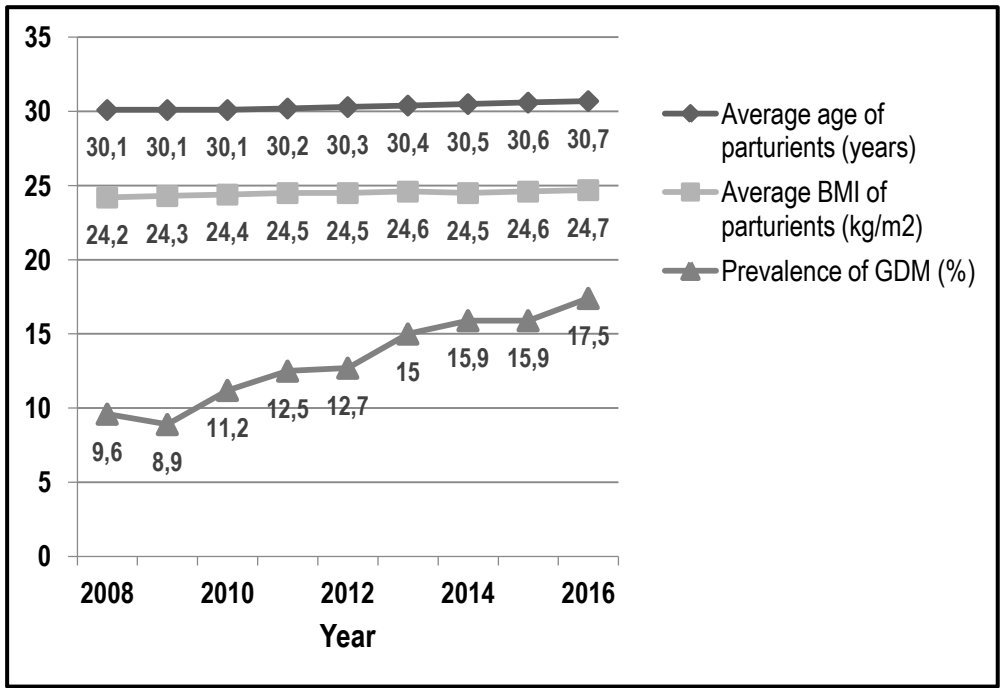
ACOG: American Congress of Obstetricians and Gynecologists; C&C: Carpenter & Coustan; CDA: Canadian Diabetes Association; EASD: European Association for the Study of Diabetes; GCT: glucose challenge test; GDM: gestational diabetes mellitus; IADPSG: International Association of the Diabetes and Pregnancy Study Groups; OGTT: oral glucose tolerance test; NDDG: National Diabetes Data Group; NICE: National Institute for Health and Care Excellence; No.: number; WHO: World Health Organization.

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.

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

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/m^2) 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/m^2). 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.

Clinical measures	WHO (1998)	NCEP ATP III (2001)	IDF (2005)
Central obesity	waist-to-hip ratio > 0.85 and/or BMI > 30 kg/m^2	WC ≥ 88 cm	Increased (population-specific) WC
fP-Gluc, mmol/L	IGT, IFG or T2DM	≥ 6.1 or diabetes	≥ 5.6 or diabetes
BP, mmHg	≥ 140/90	≥ 130/85	≥ 130/85 or treatment for hypertension
TGs, mmol/L	≥ 1.7	≥ 1.7	≥ 1.7 or treatment for this lipid abnormality
HDL-C, mmol/L	≤ 1.0	< 1.3	< 1.3 or treatment for this lipid abnormality
Other	Microalbuminuria	—	—
Definition	**IGT, IFG, T2DM**, or lowered insulin sensitivity **+ any 2 of the components**	**≥ any 3 of the components**	**Increased WC + any 2 of the components**

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.

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

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

financial burden in public health care. The Finnish National Institute for Health and Welfare has estimated that 1.4–7% of all the expenses of public health care result from obesity. In 2011, obesity and its comorbidities cost approximately 330 million euros (Finnish National Institute for Health and Welfare. 2015).

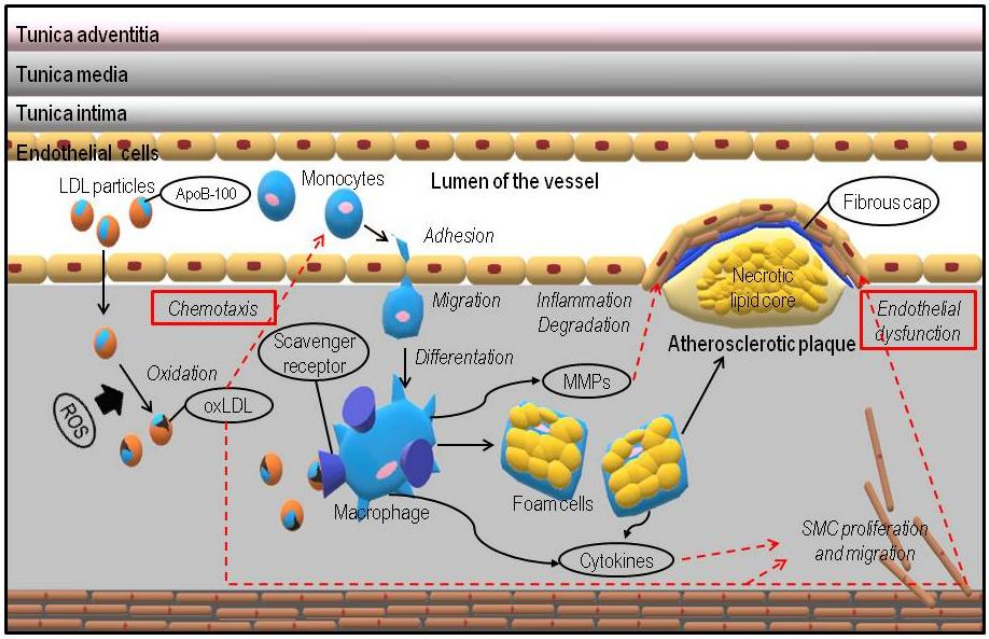
Because of the health risks and significant increase in prevalence worldwide in recent decades, obesity has also become a major global health challenge. In contrast to other major global risks, there is little evidence of successful population-level intervention strategies to reduce the increasing incidence (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 anti-oxidant 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 intimae of arterial walls. In the intimae 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

risk factors present. 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).

Variable of low-grade inflammation	Synthesized mainly by	Function
hsCRP	hepatocytes	Acute-phase reactant: elevated in response to acute infections, inflammatory conditions and trauma Predictive value in T2DM, MetS, increased carotid intima-media thickness, CVD
MMP-8	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	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
MMP-9	leukocytes, fibroblasts, macrophages, epithelial and endothelial cells	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
TIMP-1	cardiac myocytes, fibroblasts, endothelial and smooth-muscle cells, monocytes and macrophages	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

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

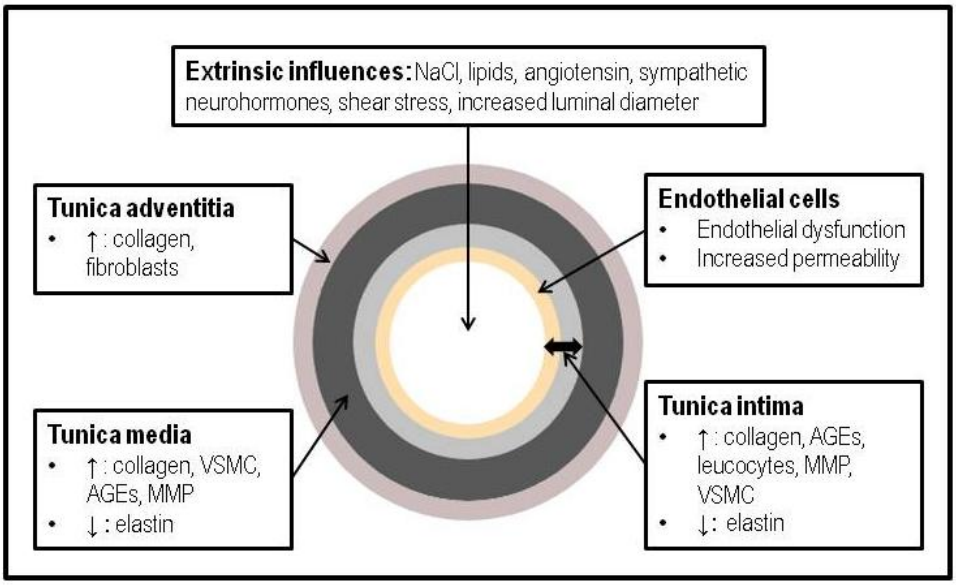
myocardial infarction (Maseri *et al.* 1978). So far, many well-established conventional CVD risk components, such as hypercholesterolemia, smoking, hypertension, obesity, microalbuminuria, IR and T2DM have been associated with endothelial dysfunction (Anderson *et al.* 1995, Goodfellow *et al.* 1996, 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 mediasclerosis 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 inter-dependent 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).



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).

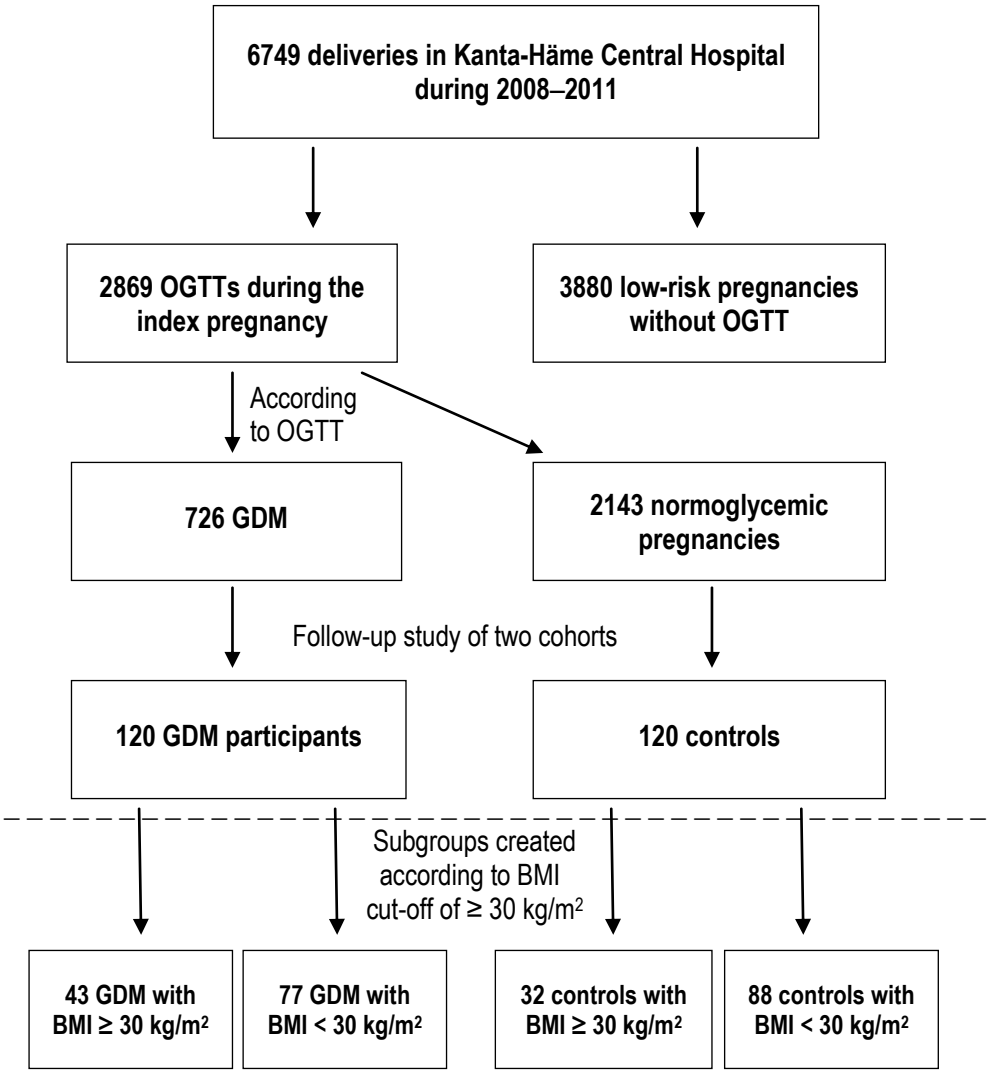


Figure 4. Flow chart describing the recruitment of two cohorts in Studies I–III. Further, under the dashed line it illustrates the division of four subgroups in Studies II & III. In Finland, GDM screening using a 75-g OGTT is offered to all pregnant women except those who are at the lowest risk: primiparous women < 25 years old, BMI ≤ 25 kg/m^2 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/m^2 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/m^2. The BMI cut-off of 27 kg/m^2 represents the average BMI of among Finnish women relatively well (26.8 kg/m^2 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/m^2, but also 27 kg/m^2 with comorbidity (Colman. 2012). In Studies II and III, obesity was classified according to the WHO recommendation as BMI of ≥ 30 kg/m^2 (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.

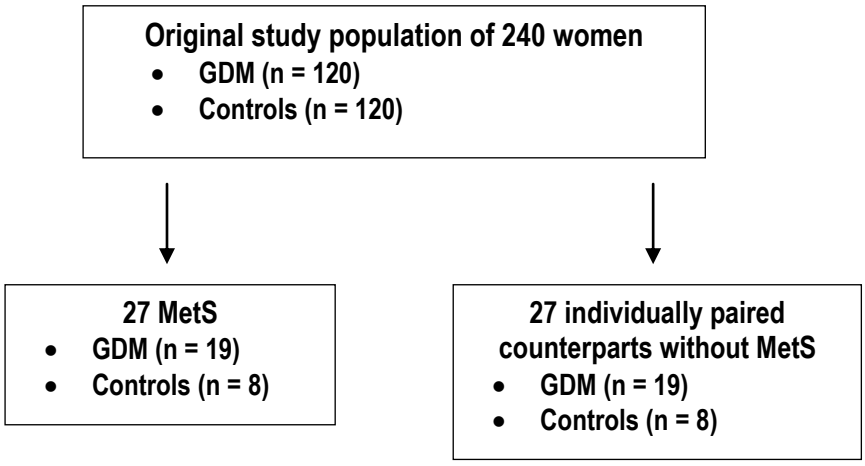


Figure 5. Flow chart illustrating the study population in the cross-sectional Study IV. Besides GDM status, the matching parameters between MetS women and individually paired counterparts without the syndrome were age and serum concentrations of both TC and LDL-C. There was no significant difference 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/m^2).

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 immunosorbent 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 H_2SO_4 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 [fP -Insu (mU/L) × fP-Gluc (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 caffeinated 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

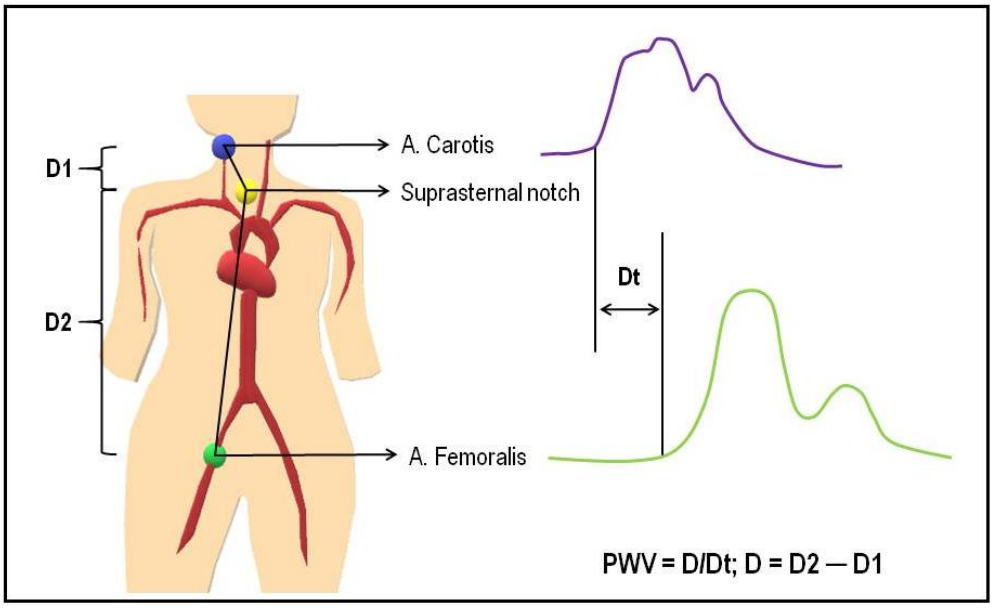
Radial artery pulse waves were recorded non-invasively with an arterial tonometer (HDI/PulseWaveTMCR-2000, Hypertension Diagnostics, Inc., Eagan, Minnesota, USA) and the procedure involves the use of a modified Windkessel pulse-contour method (Cohn *et al.* 1995). Blood volume inertia and systemic vascular 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 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.



D: distance; Dt: Time delay/transit time; PWV: pulse wave velocity

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.

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

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.

	GDM n = 120		Controls n = 120		
	Mean	**SD**	**Mean**	**SD**	**p value**
Follow-up study					
Average time since delivery (years)	3.7	1.0	3.7	0.9	0.818
Age (years)	35.8	4.4	35.9	4.6	0.854
Smoking status					0.018
Current, n (%)	24 (20.0%)		12 (10.0%)		
Former, n (%)	45 (37.5%)		37 (30.8%)		
Never, n (%)	51 (42.5%)		71 (59.2%)		
Pack-years of smoking	3.8	6.0	2.4	4.6	0.012
BMI, kg/m^2	28.3	5.0	27.5	5.4	0.069
WC, cm	96.8	13.0	92.5	12.6	0.009
Systolic BP, mmHg	122.4	12.5	119.0	11.5	0.034
Diastolic BP, mmHg	73.5	9.0	71.8	8.7	0.176
Heart rate, beats per minute	65.9	9.1	63.8	9.6	0.017
Clinical chemistry					
Leukocytes, 10^9/L	5.8	1.6	5.2	1.4	0.008
Hemoglobin, g/L	133.2	9.3	128.6	12.9	0.001
Platelets, 10^9/L	241.9	58.2	244.0	52.5	0.692
ALAT, U/L	22.8	17.4	19.7	10.5	0.116
Creatinine, µmol/L	66.6	7.7	64.5	7.8	0.048
Fibrinogen, g/L	3.4	0.9	3.2	1.0	0.096
U-AlbCre, mg/mmol	0.67	0.5	0.57	0.3	0.070

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.

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

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.

Variable of glucose metabolism	GDM n = 119		Controls n = 120		p value
	Mean	SD	Mean	SD	
fP-Gluc, mmol/L	5.61	0.70	5.26	0.33	< 0.001
fP-Insu, mU/L	5.21	3.63	4.63	3.60	0.087
HOMA-IR	1.30	0.91	1.09	0.89	0.022
HbA1c, mmol/mol	34.9	3.28	33.8	1.84	0.012

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.

	GDM n = 119		Controls n = 120		
Lipids	**Mean**	**SD**	**Mean**	**SD**	**p value**
TC, mmol/L	4.71	0.86	4.59	0.83	0.329
HDL-C, mmol/L	1.51	0.31	1.56	0.33	0.450
LDL-C, mmol/L	2.94	0.67	2.84	0.64	0.295
TGs, mmol/L	1.10	0.63	0.85	0.35	< 0.001
non-HDL-C, mmol/L	3.21	0.85	3.03	0.75	0.167
oxLDL, U/L	42.4	14.4	39.7	13.8	0.120

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.

Variable of low-grade inflammation	GDM n = 119		Controls n = 120		p value
	Mean	SD	Mean	SD	
hsCRP, mg/L	2.51	3.69	2.50	4.19	0.582
MMP-8, ng/mL	27.8	16.1	32.8	20.8	0.082
MMP-9, ng/mL	384.3	143.5	392.2	138.0	0.667
TIMP-1, ng/mL	102.8	29.7	94.6	24.5	0.020

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.

Determinant of arterial stiffness	GDM n = 120		Controls n = 120		p value
	Mean	SD	Mean	SD	
C1, mL/mmHg×10	15.1	3.51	15.9	3.36	0.092
C2, mL/mmHg×100	8.44	3.08	8.60	3.20	0.681
PWV, m/s	6.44	0.83	6.17	0.74	0.009
Systolic cBP, mmHg	110.6	12.4	107.5	11.5	0.061
Diastolic cBP, mmHg	74.5	9.11	72.7	8.78	0.123

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/m^2. When using this cut-off point, there were 122 women in the "obese" group (BMI ≥ 27 kg/m^2); 65 GDM and 57 control participants. The "non-obese" group (BMI < 27 kg/m^2; n = 118) consisted of 55 GDM and 63 control participants. In Studies II and III, obesity was classified as BMI of ≥ 30 kg/m^2. Altogether, there were 75 women in the obese group (BMI ≥ 30 kg/m^2); 43 GDM and 32 control participants. The non-obese group (BMI < 30 kg/m^2; 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/m^2 are shown in Table 13, while those with a BMI cut-off of 27 kg/m^2 are presented in Study I.

Table 13. Results of follow-up study in four subgroups.

	GDM				Control				
	BMI ≥ 30 n = 43		BMI < 30 n = 77		BMI ≥ 30 n = 32		BMI < 30 n = 88		Overall
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p value
Follow-up study									
Age, years	35.8	4.4	35.9	4.5	36.3	5.4	35.6	4.4	0.913
BMI, kg/m²	33.7	3.1	25.3	2.7	34.4	4.6	25.0	2.8	< 0.001
Pack years of smoking	4.8	8.3	3.2	4.3	4.4	6.9	1.7	3.3	0.006
Weight loss during lifetime, kg	34.1	29.3	15.5	19.9	29.3	28.1	12.1	16.5	< 0.001
MetS, n (%)	11 (25.6%)		8 (10.4%)		7 (21.9%)		1 (1.1%)		< 0.001
Clinical chemistry and immunoassays									
fP-Gluc, mmol/L	5.7	0.4	5.6	0.8	5.3	0.4	5.2	0.3	< 0.001
fP-Insu, mU/L	6.7	4.5	4.5	2.8	7.7	5.2	3.5	1.9	< 0.001
HOMA-IR	1.7	1.1	1.1	0.7	1.8	1.3	0.8	0.4	< 0.001
HbA1c, mmol/mol	34.8	2.4	34.9	3.7	33.8	1.5	33.8	2.0	0.024
TC, mmol/L	4.87	0.88	4.63	0.85	4.88	0.98	4.48	0.75	0.037
HDL-C, mmol/L	1.45	0.29	1.54	0.32	1.49	0.33	1.58	0.33	0.134
LDL-C, mmol/L	3.10	0.68	2.86	0.65	3.08	0.73	2.75	0.58	0.009
TG, mmol/L	1.29	0.75	1.00	0.53	1.05	0.42	0.78	0.30	< 0.001
non-HDL-C, mmol/L	3.34	1.02	3.09	0.80	3.39	0.85	2.90	0.67	0.005
oxLDL, U/L	44.8	14.6	41.3	14.1	42.2	15.1	38.9	13.3	0.144
hsCRP, mg/L	3.3	3.7	2.1	3.7	4.2	4.9	1.9	3.7	0.015
MMP-8, ng/mL	27.8	11.5	28.0	18.3	37.1	23.5	31.1	19.7	0.090
MMP-9, ng/mL	418.5	132.6	365.2	147.5	419.4	118.4	382.0	144.6	0.128
TIMP-1, ng/mL	105.3	32.2	101.9	28.3	96.2	19.5	94.1	26.3	0.110
Measurements of arterial function									
C1, mL/mmHg×10	14.4	3.5	15.5	3.5	15.3	3.1	16.1	3.4	0.080
C2, mL/mmHg×100	8.6	2.7	8.3	3.3	8.8	3.4	8.5	3.1	0.956
PWV, m/s	6.8	0.8	6.2	0.7	6.5	0.8	6.0	0.7	< 0.001
Systolic cBP, mmHg	111.1	25.2	104.0	19.5	111.1	16.1	101.4	20.0	0.025
Diastolic cBP, mmHg	76.1	17.6	69.3	13.1	74.7	9.7	68.7	14.6	0.013

BMI: body mass index; cBP: central blood pressure; C1: large arterial compliance; C2: small arterial compliance; fP-Gluc: fasting plasma glucose; fP-Insu; fasting plasma insulin; GDM: gestational diabetes mellitus; HbA1c: glycosylated hemoglobin A1c; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; MetS: metabolic syndrome; MMP: matrix metalloproteinase; oxLDL: oxidized low-density lipoprotein; PWV: pulse wave velocity; TC: total cholesterol; TG: triglycerides; TIMP: tissue inhibitor of metalloproteinase

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.

	MetS n = 27		Paired counterparts n = 27		
	Mean	**SD**	**Mean**	**SD**	**p value**
Matching parameter					
Age, years	36.8	4.7	36.6	4.5	0.880
Previous GDM, n (%)	19 (70%)		19 (70%)		1.000
TC, mmol/L	5.1	1.2	5.2	0.9	0.851
LDL-C, mmol/L	3.4	0.9	3.3	0.8	0.768
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/m^2	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, 10^9/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, 10^9/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 15. Arterial compliance, PWV and cBPs in the MetS women and their individually paired counterparts without the syndrome.

Determinant of arterial stiffness	MetS n = 27		Paired counterparts n = 27		p value
	Mean	SD	Mean	SD	
C1, mL/mmHg×10	15.1	8.0	16.1	4.4	0.034
C2, mL/mmHg×100	7.1	2.5	9.3	3.2	0.010
PWV, m/s	7.1	2.5	6.5	1.1	0.037
Systolic cBP, mmHg	120.9	12.2	111.5	16.0	0.031
Diastolic cBP, mmHg	81.3	8.5	74.1	11.2	0.035

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/m^2, 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™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® 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 β-cells, a process known as compensatory β-cell function. At first, obesity-induced IR leads to increased levels of insulin, but if the condition is prolonged or worsens, β-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 oxLDL. 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 (Tziomalos *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, Tziomalos *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/m^2, because obesity is often classified as BMI of ≥ 30 kg/m^2 (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/m^2 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/m^2 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/m^2, 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 disappointingly 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).

7 SUMMARY AND CONCLUSIONS

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 follow-up 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.

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9 REFERENCES

Adams TD, Davidson LE, Litwin SE, Kim J, Kolotkin RL, Nanjee MN, Gutierrez JM, Frogley SJ, Ibele AR, Brinton EA, Hopkins PN, McKinlay R, Simper SC & Hunt SC. (2017) Weight and Metabolic Outcomes 12 Years after Gastric Bypass. N Engl J Med 377(12): 1143-1155.

Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, Wang J, Wilkinson IB, Williams B & Vlachopoulos C. (2007) Central Blood Pressure Measurements and Antihypertensive Therapy. A Consensus Document. Hypertension 50: 154-160.

Agarwal MM. (2015) Gestational diabetes mellitus: An update on the current international diagnostic criteria. World J Diabetes 6(6): 782-791.

Aguilar M, Bhuket T, Torres S, Liu B & Wong RJ. (2015) Prevalence of the metabolic syndrome in the United States, 2003-2012. JAMA 313(19): 1973-1974.

Ajala O, Jensen LA, Ryan E & Chik C. (2015) Women with a history of gestational diabetes on long-term follow up have normal vascular function despite more dysglycemia, dyslipidemia and adiposity. Diabetes Res Clin Pract 110(3): 309-314.

Akgöl E, Abusoglu S, Gun FD & Unlu A. (2017) Prevalence of gestational diabetes mellitus according to the different criterias. Turk J Obstet Gynecol 14(1): 18-22.

Akinci B, Celtik A, Genc S, Yener S, Demir T, Secil M, Kebapcilar L & Yesil S. (2011) Evaluation of postpartum carbohydrate intolerance and cardiovascular risk factors in women with gestational diabetes. Gynecol Endocrinol 27(5): 361-367.

Albareda M, Caballero A, Badell G, Rodriguez-Espinosa J, Ordonez-Llanos J, de Leiva A & Corcoy R. (2005) Metabolic syndrome at follow-up in women with and without gestational diabetes mellitus in index pregnancy. Metabolism 54(8): 1115-1121.

Alberti KG & Zimmet PZ. (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15(7): 539-553.

American Diabetes Association. (2003) Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 26 Suppl 1: S5-20.

American Diabetes Association. (2014) Diagnosis and classification of diabetes mellitus. Diabetes Care 37 Suppl 1: S81-90.

American Diabetes Association. (2016) Standards of Medical Care in Diabetes-2016: Summary of Revisions. Diabetes Care 39 Suppl 1: S4-5.

American Diabetes Association. (2017) 2. Classification and Diagnosis of Diabetes. Diabetes Care 40(Suppl 1): S11-S24.

Anastasiou E, Lekakis JP, Alevizaki M, Papamichael CM, Megas J, Souvatzoglou A & Stamatelopoulos SF. (1998) Impaired endothelium-dependent vasodilatation in women with previous gestational diabetes. Diabetes Care 21(12): 2111-2115.

Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP & Ganz P. (1995) The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 332(8): 488-493.

Antuna-Puente B, Disse E, Rabasa-Lhoret R, Laville M, Capeau J & Bastard JP. (2011) How can we measure insulin sensitivity/resistance? Diabetes Metab 37(3): 179-188.

Aroda VR, Knowler WC, Crandall JP, Perreault L, Edelstein SL, Jeffries SL, Molitch ME, Pi-Sunyer X, Darwin C, Heckman-Stoddard BM, Temprosa M, Kahn SE, Nathan DM & Diabetes Prevention Program Research Group. (2017) Metformin for diabetes prevention: insights gained from the Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study. Diabetologia : [Epub ahead of print].

Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R & Levy BI. (1995) Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension 26(3): 485-490.

Avalos GE, Owens LA, Dunne F & ATLANTIC DIP Collaborators. (2013) Applying current screening tools for gestational diabetes mellitus to a European population: is it time for change? Diabetes Care 36(10): 3040-3044.

Balkau B, Charles MA, Drivsholm T, Borch-Johnsen K, Wareham N, Yudkin JS, Morris R, Zavaroni I, van Dam R, Feskins E, Gabriel R, Diet M, Nilsson P, Hedblad B & European Group For The Study Of Insulin Resistance (EGIR). (2002) Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. Diabetes Metab 28(5): 364-376.

Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM & Friedman JE. (2007) Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care 30 Suppl 2: S112-9.

Bauduceau B, Vachey E, Mayaudon H, Burnat P, Dupuy O, Garcia C, Ceppa F & Bordier L. (2007) Should we have more definitions of metabolic syndrome or simply take waist measurement? Diabetes Metab 33(5): 333-339.

Bellamy L, Casas JP, Hingorani AD & Williams D. (2009) Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet 373(9677): 1773-1779.

Benhalima K, Leuridan L, Calewaert P, Devlieger R, Verhaeghe J & Mathieu C. (2014) Glucose intolerance after a recent history of gestational diabetes. Int J Endocrinol 2014: 727652.

Ben-Haroush A, Yogev Y & Hod M. (2004) Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabet Med 21(2): 103-113.

Berg G, Miksztowicz V & Schreier L. (2011) Metalloproteinases in metabolic syndrome. Clin Chim Acta 412(19-20): 1731-1739.

Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD & Lusis AJ. (1995) Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 91(9): 2488-2496.

Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, Moore SC, Tobias GS, Anton-Culver H, Freeman LB, Beeson WL, Clipp SL, English DR, Folsom AR, Freedman DM, Giles G, Hakansson N, Henderson KD, Hoffman-Bolton J, Hoppin JA, Koenig KL, Lee IM, Linet MS, Park Y, Pocobelli G, Schatzkin A, Sesso HD, Weiderpass E, Willcox BJ, Wolk A, Zeleniuch-Jacquotte A, Willett WC & Thun MJ. (2010) Body-mass index and mortality among 1.46 million white adults. N Engl J Med 363(23): 2211-2219.

Bertoluci MC & Rocha VZ. (2017) Cardiovascular risk assessment in patients with diabetes. Diabetol Metab Syndr 9: 25.

Bezie Y, Lamaziere JM, Laurent S, Challande P, Cunha RS, Bonnet J & Lacolley P. (1998) Fibronectin expression and aortic wall elastic modulus in spontaneously hypertensive rats. Arterioscler Thromb Vasc Biol 18(7): 1027-1034.

Bhatia LS, Curzen NP, Calder PC & Byrne CD. (2012) Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? Eur Heart J 33(10): 1190-1200.

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

diabetic people: a population-based retrospective cohort study. Lancet 368(9529): 29-36.

Borch-Johnsen K & Wareham N. (2010) The rise and fall of the metabolic syndrome. Diabetologia 53(4): 597-599.

Borodulin K, Levälahti E, Saarikoski L, Lund L, Juolevi A, Grönholm M, Jula A, Laatikainen T, Männistö S, Peltonen M, Salomaa V, Sundvall J, Taimi M, Virtanen S & Vartiainen E. (2014) Kansallinen FINRISKI 2012 - terveystutkimus - Osa 2: Tutkimuksen taulukkoliite. Available online at: http://urn.fi/URN:ISBN:978-952-302-054-2. Juvenes Print -Suomen Yliopistopaino Oy, Tampere 22/2013.

Bowie A, Owens D, Collins P, Johnson A & Tomkin GH. (1993) Glycosylated low density lipoprotein is more sensitive to oxidation: implications for the diabetic patient? Atherosclerosis 102(1): 63-67.

Brew K & Nagase H. (2010) The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity. Biochim Biophys Acta 1803(1): 55-71.

Briet M, Boutouyrie P, Laurent S & London GM. (2012) Arterial stiffness and pulse pressure in CKD and ESRD. Kidney Int 82(4): 388-400.

Buchanan TA & Xiang AH. (2005) Gestational diabetes mellitus. J Clin Invest 115(3): 485-491.

Cameron AJ, Zimmet PZ, Shaw JE & Alberti KG. (2009) The metabolic syndrome: in need of a global mission statement. Diabet Med 26(3): 306-309.

Carpenter MW & Coustan DR. (1982) Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol 144(7): 768-773.

Carrington ER, Shuman CR & Reardon HS. (1957) Evaluation of the prediabetic state during pregnancy. Obstet Gynecol 9: 664-669.

Castro Dufourny I, Herranz de la Morena L, Martin Borge V & Pallardo Sanchez LF. (2009) Metabolic syndrome and insulin resistance: prevalence in women with prior gestational diabetes, using two different classifications. Rev Clin Esp 209(2): 61-66.

Catalano PM, Huston L, Amini SB & Kalhan SC. (1999) Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. Am J Obstet Gynecol 180(4): 903-916.

Catalano PM, Kirwan JP, Haugel-de Mouzon S & King J. (2003) Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. J Nutr 133(5 Suppl 2): 1674S-1683S.

Catalano PM, Tyzbir ED, Roman NM, Amini SB & Sims EA. (1991) Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. Am J Obstet Gynecol 165(6 Pt 1): 1667-1672.

Catapano AL, Maggi FM & Tragni E. (2000) Low density lipoprotein oxidation, antioxidants, and atherosclerosis. Curr Opin Cardiol 15(5): 355-363.

Catov JM & Margerison-Zilko C. (2016) Pregnancy as a window to future health: short-term costs and consequences. Am J Obstet Gynecol 215(4): 406-407.

Cavusoglu E, Ruwende C, Chopra V, Yanamadala S, Eng C, Clark LT, Pinsky DJ & Marmur JD. (2006) Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction. Am Heart J 151(5): 1101.e1-1101.e8.

Cereda E, Malavazos AE, Caccialanza R, Rondanelli M, Fatati G & Barichella M. (2011) Weight cycling is associated with body weight excess and abdominal fat accumulation: a cross-sectional study. Clin Nutr 30(6): 718-723.

Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH & Hu FB. (2009) Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA 301(20): 2129-2140.

Chang Y & Robidoux J. (2017) Dyslipidemia management update. Curr Opin Pharmacol 33: 47-55.

Chenillot O, Henny J, Steinmetz J, Herbeth B, Wagner C & Siest G. (2000) High sensitivity C-reactive protein: biological variations and reference limits. Clin Chem Lab Med 38(10): 1003-1011.

Cheung YF. (2010) Arterial stiffness in the young: assessment, determinants, and implications. Korean Circ J 40(4): 153-162.

Chevalier N, Fenichel P, Giaume V, Loizeau S, Bongain A, Daideri G, Brucker-Davis F & Hieronimus S. (2011) Universal two-step screening strategy for gestational diabetes has weak relevance in French Mediterranean women: should we simplify the screening strategy for gestational diabetes in France? Diabetes Metab 37(5): 419-425.

Cho Y, Lee SG, Jee SH & Kim JH. (2015) Hypertriglyceridemia is a major factor associated with elevated levels of small dense LDL cholesterol in patients with metabolic syndrome. Ann Lab Med 35(6): 586-594.

Cho YM, Kim TH, Lim S, Choi SH, Shin HD, Lee HK, Park KS & Jang HC. (2009) Type 2 diabetes-associated genetic variants discovered in the recent genome-wide association studies are related to gestational diabetes mellitus in the Korean population. Diabetologia 52(2): 253-261.

Choi SM, Tucker DF, Gross DN, Easton RM, DiPilato LM, Dean AS, Monks BR & Birnbaum MJ. (2010) Insulin regulates adipocyte lipolysis via an Akt-independent signaling pathway. Mol Cell Biol 30(21): 5009-5020.

Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, Armitage J & Baigent C. (2008) Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. Lancet 371(9607): 117-125.

Clark HD, Graham ID, Karovitch A & Keely EJ. (2009) Do postal reminders increase postpartum screening of diabetes mellitus in women with gestational diabetes mellitus? A randomized controlled trial. Am J Obstet Gynecol 200(6): 634 (e1-7).

Cohn JN. (1999) Vascular wall function as a risk marker for cardiovascular disease. J Hypertens Suppl 17(5): S41-4.

Cohn JN, Finkelstein S, McVeigh G, Morgan D, LeMay L, Robinson J & Mock J. (1995) Noninvasive pulse wave analysis for the early detection of vascular disease. Hypertension 26(3): 503-508.

Colman E. (2012) Food and Drug Administration's Obesity Drug Guidance Document: a short history. Circulation 125(17): 2156-2164.

Cortez-Cooper MY, Supak JA & Tanaka H. (2003) A new device for automatic measurements of arterial stiffness and ankle-brachial index. Am J Cardiol 91(12): 1519-22, A9.

Cossrow N & Falkner B. (2004) Race/ethnic issues in obesity and obesity-related comorbidities. J Clin Endocrinol Metab 89(6): 2590-2594.

Craig VJ, Zhang L, Hagood JS & Owen CA. (2015) Matrix Metalloproteinases as Therapeutic Targets for Idiopathic Pulmonary Fibrosis. Am J Respir Cell Mol Biol 53(5): 585-600.

Dabelea D, Snell-Bergeon JK, Hartsfield CL, Bischoff KJ, Hamman RF, McDuffie RS & Kaiser Permanente of Colorado GDM Screening Program. (2005) Increasing prevalence of gestational diabetes mellitus (GDM) over time and by birth cohort: Kaiser Permanente of Colorado GDM Screening Program. Diabetes Care 28(3): 579-584.

Davenport MH, Goswami R, Shoemaker JK & Mottola MF. (2012) Influence of hyperglycemia during and after pregnancy on postpartum vascular function. Am J Physiol Regul Integr Comp Physiol 302(6): R768-75.

Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W & Auwerx J. (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20(3): 284-287.

DeFronzo RA & Abdul-Ghani MA. (2011) Preservation of beta-cell function: the key to diabetes prevention. J Clin Endocrinol Metab 96(8): 2354-2366.

DeFronzo RA, Tobin JD & Andres R. (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237(3): E214-23.

Derbent A, Kargili A, Koca C, Gumus II, Sevgili S, Simavli S, Karakurt F & Turhan NO. (2011) Serum platelet-activating factor acetylhydrolase activity: relationship with metabolic syndrome in women with history of gestational diabetes mellitus. Gynecol Endocrinol 27(2): 128-133.

Di Cianni G, Ghio A, Resi V & Volpe L. (2010) Gestational diabetes mellitus: an opportunity to prevent type 2 diabetes and cardiovascular disease in young women. Womens Health (Lond) 6(1): 97-105.

Di Cianni G, Lencioni C, Volpe L, Ghio A, Cuccuru I, Pellegrini G, Benzi L, Miccoli R & Del Prato S. (2007) C-reactive protein and metabolic syndrome in women with previous gestational diabetes. Diabetes Metab Res Rev 23(2): 135-140.

Diabetes. Current Care Guidelines. (2018) Diabetes (online). Current Care Guidelines. Working group set up by Finnish Medical Society Duodecim, the Finnish Society of Internal Medicine and the Medical Advisory Board of the Finnish Diabetes Association. Helsinki: The Finnish Medical Society Duodecim, 2018 (referred March 1, 2018). Available online at: www.kaypahoito.fi.

Dongerkery SP, Schroeder PR & Shomali ME. (2017) Insulin and Its Cardiovascular Effects: What Is the Current Evidence? Curr Diab Rep 17(12): 120.

Duncan M. (1882) On puerperal diabetes. Trans Obstet Soc Lond 24: 256-285.

Durnwald C. (2015) Gestational diabetes: Linking epidemiology, excessive gestational weight gain, adverse pregnancy outcomes, and future metabolic syndrome. Semin Perinatol 39(4): 254-258.

Dwivedi A, Al'Aref SJ, Lin FY & Min JK. (2018) Evaluation of Atherosclerotic Plaque in Non-invasive Coronary Imaging. Korean Circ J 48(2): 124-133.

Edouard DA, Pannier BM, London GM, Cuche JL & Safar ME. (1998) Venous and arterial behavior during normal pregnancy. Am J Physiol 274(5 Pt 2): H1605-12.

Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V & Patel A. (2015) Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. JAMA 313(6): 603-615.

Ervin RB. (2009) Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003-2006. Natl Health Stat Report (13): 1-7.

Fadl H, Magnuson A, Ostlund I, Montgomery S, Hanson U & Schwarcz E. (2014) Gestational diabetes mellitus and later cardiovascular disease: a Swedish population based case-control study. BJOG 121(12): 1530-1536.

Faxon DP, Fuster V, Libby P, Beckman JA, Hiatt WR, Thompson RW, Topper JN, Annex BH, Rundback JH, Fabunmi RP, Robertson RM, Loscalzo J & American Heart Association. (2004) Atherosclerotic Vascular Disease Conference: Writing Group III: pathophysiology. Circulation 109(21): 2617-2625.

Feng D, Zhou Y, Xia M & Ma J. (2011) Folic acid inhibits lipopolysaccharide-induced inflammatory response in RAW264.7 macrophages by suppressing MAPKs and NF-kappaB activation. Inflamm Res 60(9): 817-822.

Finkelstein SM, Collins VR & Cohn JN. (1988) Arterial vascular compliance response to vasodilators by Fourier and pulse contour analysis. Hypertension 12(4): 380-387.

Finnish National Institute for Health and Welfare. (2015) Lihavuus lukuina. Finnish National Institute for Health and Welfare, 2015. Available online at: https://www.thl.fi/fi/tutkimus-ja-asiantuntijatyo/hankkeet-ja-ohjelmat/kansallinenlihavuusohjelma-20122015/lihavuus-lukuina.

Finnish National Institute for Health and Welfare. (2016) Diabeteksen kustannukset. Finnish National Institute for Health and Welfare, 2016. Available online at: https://www.thl.fi/fi/web/kansantaudit/diabetes/diabeteksen-kustannukset.

Flegal KM, Carroll MD, Kit BK & Ogden CL. (2012) Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 307(5): 491-497.

Förstermann U, Xia N & Li H. (2017) Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circ Res 120(4): 713-735.

Fraser A, Nelson SM, Macdonald-Wallis C, Cherry L, Butler E, Sattar N & Lawlor DA. (2012) Associations of pregnancy complications with calculated cardiovascular disease risk and cardiovascular risk factors in middle age: the Avon Longitudinal Study of Parents and Children. Circulation 125(11): 1367-1380.

Fraser A, Tilling K, Macdonald-Wallis C, Hughes R, Sattar N, Nelson SM & Lawlor DA. (2011) Associations of gestational weight gain with maternal body mass index, waist circumference, and blood pressure measured 16 y after

pregnancy: the Avon Longitudinal Study of Parents and Children (ALSPAC). Am J Clin Nutr 93(6): 1285-1292.

Fruchart JC, Nierman MC, Stroes ES, Kastelein JJ & Duriez P. (2004) New risk factors for atherosclerosis and patient risk assessment. Circulation 109(23 Suppl 1): III15-9.

Fruhbeck G, Toplak H, Woodward E, Yumuk V, Maislos M, Oppert JM & Executive Committee of the European Association for the Study of Obesity. (2013) Obesity: the gateway to ill health - an EASO position statement on a rising public health, clinical and scientific challenge in Europe. Obes Facts 6(2): 117-120.

Furie MB & Mitchell RN. (2012) Plaque attack: one hundred years of atherosclerosis in The American Journal of Pathology. Am J Pathol 180(6): 2184-2187.

Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK & Montori VM. (2007) Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol 49(4): 403-414.

Ge JY, Li XL, Zhang HF, Xu Q, Tong M & Wang JG. (2008) Elasticity indices of large and small arteries in relation to the metabolic syndrome in Chinese. Am J Hypertens 21(2): 143-147.

Gestational diabetes. Current Care Guidelines. (2013) Gestational diabetes (online). Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim, the Medical Advisory Board of the Finnish Diabetes Association and the Finnish Gynecological Association, 2013 (Referred November 14, 2017). Available online at: www.kaypahoito.fi.

Gillett MJ. (2009) International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes: Diabetes Care 2009; 32(7): 1327-1334. Clin Biochem Rev 30(4): 197-200.

Gilmore LA, Klempel-Donchenko M & Redman LM. (2015) Pregnancy as a window to future health: Excessive gestational weight gain and obesity. Semin Perinatol 39(4): 296-303.

Ginsberg HN. (1998) Lipoprotein physiology. Endocrinol Metab Clin North Am 27(3): 503-519.

Giroud D, Li JM, Urban P, Meier B & Rutishauer W. (1992) Relation of the site of acute myocardial infarction to the most severe coronary arterial stenosis at prior angiography. Am J Cardiol 69(8): 729-732.

Global BMI Mortality C, Di Angelantonio E, Bhupathiraju S, Wormser D, Gao P, Kaptoge S, Berrington de Gonzalez A, Cairns BJ, Huxley R, Jackson C, Joshy

G, Lewington S, Manson JE, Murphy N, Patel AV, Samet JM, Woodward M, Zheng W, Zhou M, Bansal N, Barricarte A, Carter B, Cerhan JR, Smith GD, Fang X, Franco OH, Green J, Halsey J, Hildebrand JS, Jung KJ, Korda RJ, McLerran DF, Moore SC, O'Keeffe LM, Paige E, Ramond A, Reeves GK, Rolland B, Sacerdote C, Sattar N, Sofianopoulou E, Stevens J, Thun M, Ueshima H, Yang L, Yun YD, Willeit P, Banks E, Beral V, Chen Z, Gapstur SM, Gunter MJ, Hartge P, Jee SH, Lam TH, Peto R, Potter JD, Willett WC, Thompson SG, Danesh J & Hu FB. (2016) Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet 388(10046): 776-786.

Gobl CS, Bozkurt L, Yarragudi R, Prikoszovich T, Tura A, Pacini G, Koppensteiner R & Kautzky-Willer A. (2014) Biomarkers of endothelial dysfunction in relation to impaired carbohydrate metabolism following pregnancy with gestational diabetes mellitus. Cardiovasc Diabetol 13(1): 138.

Goldstein JL, Ho YK, Basu SK & Brown MS. (1979) Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc Natl Acad Sci U S A 76(1): 333-337.

Goncalves FM, Jacob-Ferreira AL, Gomes VA, Casella-Filho A, Chagas AC, Marcaccini AM, Gerlach RF & Tanus-Santos JE. (2009) Increased circulating levels of matrix metalloproteinase (MMP)-8, MMP-9, and pro-inflammatory markers in patients with metabolic syndrome. Clin Chim Acta 403(1-2): 173-177.

Goodfellow J, Ramsey MW, Luddington LA, Jones CJ, Coates PA, Dunstan F, Lewis MJ, Owens DR & Henderson AH. (1996) Endothelium and inelastic arteries: an early marker of vascular dysfunction in non-insulin dependent diabetes. BMJ 312(7033): 744-745.

Gotto AM,Jr, Pownall HJ & Havel RJ. (1986) Introduction to the plasma lipoproteins. Methods Enzymol 128: 3-41.

Goueslard K, Cottenet J, Mariet AS, Giroud M, Cottin Y, Petit JM & Quantin C. (2016) Early cardiovascular events in women with a history of gestational diabetes mellitus. Cardiovasc Diabetol 15: 15.

Guerin AP, London GM, Marchais SJ & Metivier F. (2000) Arterial stiffening and vascular calcifications in end-stage renal disease. Nephrol Dial Transplant 15(7): 1014-1021.

Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL & Anis AH. (2009) The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health 9: 88.

Gunderson EP, Chiang V, Pletcher MJ, Jacobs DR, Quesenberry CP, Sidney S & Lewis CE. (2014) History of gestational diabetes mellitus and future risk of atherosclerosis in mid-life: the Coronary Artery Risk Development in Young Adults study. J Am Heart Assoc 3(2): e000490.

Gutch M, Kumar S, Razi SM, Gupta KK & Gupta A. (2015) Assessment of insulin sensitivity/resistance. Indian J Endocrinol Metab 19(1): 160-164.

Haffner SM, Lehto S, Rönnemaa T, Pyörälä K & Laakso M. (1998) Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 339(4): 229-234.

Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, van Hinsbergh VW, Helaakoski T, Kainulainen T, Ronka H, Tschesche H & Salo T. (1997) Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. J Biol Chem 272(50): 31504-31509.

Hannemann MM, Liddell WG, Shore AC, Clark PM & Tooke JE. (2002) Vascular function in women with previous gestational diabetes mellitus. J Vasc Res 39(4): 311-319.

HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS & Sacks DA. (2008) Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 358(19): 1991-2002.

Hashimoto J. (2014) Central hemodynamics and target organ damage in hypertension. Tohoku J Exp Med 233(1): 1-8.

Hayakawa T, Yamashita K, Tanzawa K, Uchijima E & Iwata K. (1992) Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. A possible new growth factor in serum. FEBS Lett 298(1): 29-32.

Healy B. (1990) Endothelial cell dysfunction: an emerging endocrinopathy linked to coronary disease. J Am Coll Cardiol 16(2): 357-358.

Heitritter SM, Solomon CG, Mitchell GF, Skali-Ounis N & Seely EW. (2005) Subclinical inflammation and vascular dysfunction in women with previous gestational diabetes mellitus. J Clin Endocrinol Metab 90(7): 3983-3988.

Henry OA & Beischer NA. (1991) Long-term implications of gestational diabetes for the mother. Baillieres Clin Obstet Gynaecol 5(2): 461-483.

Hess PL, Al-Khalidi HR, Friedman DJ, Mulder H, Kucharska-Newton A, Rosamond WR, Lopes RD, Gersh BJ, Mark DB, Curtis LH, Post WS, Prineas

RJ, Sotoodehnia N & Al-Khatib SM. (2017) The Metabolic Syndrome and Risk of Sudden Cardiac Death: The Atherosclerosis Risk in Communities Study. J Am Heart Assoc 6(8): e006103.

Hodes RJ, Lakatta EG & McNeil CT. (1995) Another modifiable risk factor for cardiovascular disease? Some evidence points to arterial stiffness. J Am Geriatr Soc 43(5): 581-582.

Holman RR. (1998) Assessing the potential for alpha-glucosidase inhibitors in prediabetic states. Diabetes Res Clin Pract 40 Suppl: S21-5.

Holtkamp HC, Verhoef NJ & Leijnse B. (1975) The difference between the glucose concentrations in plasma and whole blood. Clin Chim Acta 59(1): 41-49.

Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Collen D, Muls E & Van de Werf F. (2001) Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 21(5): 844-848.

Holvoet P, Vanhaecke J, Janssens S, Van de Werf F & Collen D. (1998) Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 98(15): 1487-1494.

Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwinkle E & Ballantyne CM. (2014) Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol 34(5): 1069-1077.

Hopmans TE, van Houten C, Kasius A, Kouznetsova OI, Nguyen LA, Rooijmans SV, Voormolen DN, van Vliet EO, Franx A & Koster MP. (2015) Increased risk of type II diabetes mellitus and cardiovascular disease after gestational diabetes mellitus: a systematic review. Ned Tijdschr Geneeskd 159: A8043.

Hossain P, Kawar B & El Nahas M. (2007) Obesity and diabetes in the developing world--a growing challenge. N Engl J Med 356(3): 213-215.

Hruby A & Hu FB. (2015) The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics 33(7): 673-689.

Hu H, Nakagawa T, Honda T, Yamamoto S, Nanri A, Konishi M, Okazaki H, Kuwahara K, Hori A, Nishiura C, Kashino I, Imai T, Nishihara A, Akter S, Miyamoto T, Sasaki N, Ogasawara T, Uehara A, Yamamoto M, Murakami T, Shimizu M, Eguchi M, Kochi T, Nagahama S, Tomita K, Kabe I, Mizoue T, Sone T, Dohi S & Japan Epidemiology Collaboration on Occupational Health Study Group. (2017) Metabolic Syndrome Over 4 Years Before the Onset of Cardiovascular Disease- Nested Case-Control Study. Circ J : [Epub ahead of print].

Hu J, Norman M, Wallensteen M & Gennser G. (1998) Increased large arterial stiffness and impaired acetylcholine induced skin vasodilatation in women with previous gestational diabetes mellitus. Br J Obstet Gynaecol 105(12): 1279-1287.

Hunt KJ & Schuller KL. (2007) The increasing prevalence of diabetes in pregnancy. Obstet Gynecol Clin North Am 34(2): 173-199.

Hurrle S & Hsu WH. (2017) The etiology of oxidative stress in insulin resistance. Biomed J 40(5): 257-262.

Huxley R, Barzi F & Woodward M. (2006) Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. BMJ 332(7533): 73-78.

Ijäs H, Morin-Papunen L, Keränen AK, Bloigu R, Ruokonen A, Puukka K, Ebeling T, Raudaskoski T & Vääräsmäki M. (2013) Pre-pregnancy overweight overtakes gestational diabetes as a risk factor for subsequent metabolic syndrome. Eur J Endocrinol 169(5): 605-611.

Ilanne-Parikka P, Eriksson JG, Lindström J, Hämäläinen H, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Mannelin M, Rastas M, Salminen V, Aunola S, Sundvall J, Valle T, Lahtela J, Uusitupa M, Tuomilehto J & Finnish Diabetes Prevention Study Group. (2004) Prevalence of the metabolic syndrome and its components: findings from a Finnish general population sample and the Diabetes Prevention Study cohort. Diabetes Care 27(9): 2135-2140.

Insull W,Jr. (2009) The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. Am J Med 122(1 Suppl): S3-S14.

International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva A, Hod M, Kitzmiler JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y & Schmidt MI. (2010) International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 33(3): 676-682.

International Diabetes Federation. (2006) The IDF consensus worldwide definition of the metabolic syndrome. Available online at: http://www.idf.org/metabolic-syndrome.

International Diabetes Federation. (2011) IDF Diabetes Atlas; 5th edition. Available online at: http://www.idf.org/diabetesatlas/5e/.

Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR & Groop L. (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 24(4): 683-689.

James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, LeFevre ML, MacKenzie TD, Ogedegbe O, Smith SC,Jr, Svetkey LP, Taler SJ, Townsend RR, Wright JT,Jr, Narva AS & Ortiz E. (2014) 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA 311(5): 507-520.

Jane M, Hagger M, Foster J, Ho S, Kane R & Pal S. (2017) Effects of a weight management program delivered by social media on weight and metabolic syndrome risk factors in overweight and obese adults: A randomised controlled trial. PLoS One 12(6): e0178326.

Järvelä IY, Juutinen J, Koskela P, Hartikainen AL, Kulmala P, Knip M & Tapanainen JS. (2006) Gestational diabetes identifies women at risk for permanent type 1 and type 2 diabetes in fertile age: predictive role of autoantibodies. Diabetes Care 29(3): 607-612.

Jensen LA, Chik CL & Ryan EA. (2016) Review of gestational diabetes mellitus effects on vascular structure and function. Diab Vasc Dis Res 13(3): 170-182.

Johnson CP, Baugh R, Wilson CA & Burns J. (2001) Age related changes in the tunica media of the vertebral artery: implications for the assessment of vessels injured by trauma. J Clin Pathol 54(2): 139-145.

Jones EJ, Roche CC & Appel SJ. (2009) A review of the health beliefs and lifestyle behaviors of women with previous gestational diabetes. J Obstet Gynecol Neonatal Nurs 38(5): 516-526.

Kahn R, Buse J, Ferrannini E, Stern M, American Diabetes Association & European Association for the Study of Diabetes. (2005) The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 28(9): 2289-2304.

Kalaria RN, Akinyemi R & Ihara M. (2012) Does vascular pathology contribute to Alzheimer changes? J Neurol Sci 322(1-2): 141-147.

Kamath DY, Xavier D, Sigamani A & Pais P. (2015) High sensitivity C-reactive protein (hsCRP) & cardiovascular disease: An Indian perspective. Indian J Med Res 142(3): 261-268.

Kampangkaew J, Pickett S & Nambi V. (2017) Advances in the management of dyslipidemia. Curr Opin Cardiol 32(4): 348-355.

Karadeniz M, Duran M, Akyel A, Yarlioglues M, Ocek AH, Celik IE, Kilic A, Yalcin AA, Ergun G & Murat SN. (2015) High Sensitive CRP Level Is Associated With Intermediate and High Syntax Score in Patients With Acute Coronary Syndrome. Int Heart J 56(4): 377-380.

Kärkkäinen H, Laitinen T, Heiskanen N, Saarelainen H, Valtonen P, Lyyra-Laitinen T, Vanninen E & Heinonen S. (2013) Need for insulin to control gestational diabetes is reflected in the ambulatory arterial stiffness index. BMC Pregnancy Childbirth 13: 9.

Karoli R, Siddiqi Z, Fatima J, Shukla V, Mishra PP & Khan FA. (2015) Assessment of noninvasive risk markers of subclinical atherosclerosis in premenopausal women with previous history of gestational diabetes mellitus. Heart Views 16(1): 13-18.

Kattoor AJ, Pothineni NVK, Palagiri D & Mehta JL. (2017) Oxidative Stress in Atherosclerosis. Curr Atheroscler Rep 19(11): 42.

Kaul P, Savu A, Nerenberg KA, Donovan LE, Chik CL, Ryan EA & Johnson JA. (2015) Impact of gestational diabetes mellitus and high maternal weight on the development of diabetes, hypertension and cardiovascular disease: a population-level analysis. Diabet Med 32(2): 164-173.

Kaur J. (2014) A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014: 943162.

Kelly R, Hayward C, Avolio A & O'Rourke M. (1989) Noninvasive determination of age-related changes in the human arterial pulse. Circulation 80(6): 1652-1659.

Kerenyi Z, Stella P, Bosnyak Z, Tabak AG & Tamas G. (1999) Association between central adiposity and multimetabolic syndrome in a special cohort of women with prior gestational diabetes. Diabetes Care 22(5): 876-877.

Keys A, Taylor HL & Grande F. (1973) Basal metabolism and age of adult man. Metabolism 22(4): 579-587.

Khavandi M, Duarte F, Ginsberg HN & Reyes-Soffer G. (2017) Treatment of Dyslipidemias to Prevent Cardiovascular Disease in Patients with Type 2 Diabetes. Curr Cardiol Rep 19(1): 7.

Kim C. (2010a) Gestational diabetes mellitus and risk of future maternal cardiovascular disease. Expert Rev Cardiovasc Ther 8(12): 1639-1641.

Kim C. (2010b) Gestational diabetes: risks, management, and treatment options. Int J Womens Health 2: 339-351.

Kim C, Berger DK & Chamany S. (2007) Recurrence of gestational diabetes mellitus: a systematic review. Diabetes Care 30(5): 1314-1319.

Kim C, Herman WH & Vijan S. (2007) Efficacy and cost of postpartum screening strategies for diabetes among women with histories of gestational diabetes mellitus. Diabetes Care 30(5): 1102-1106.

Kim C, Newton KM & Knopp RH. (2002) Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 25(10): 1862-1868.

Kivimäki M, Kuosma E, Ferrie JE, Luukkonen R, Nyberg ST, Alfredsson L, Batty GD, Brunner EJ, Fransson E, Goldberg M, Knutsson A, Koskenvuo M, Nordin M, Oksanen T, Pentti J, Rugulies R, Shipley MJ, Singh-Manoux A, Steptoe A, Suominen SB, Theorell T, Vahtera J, Virtanen M, Westerholm P, Westerlund H, Zins M, Hamer M, Bell JA, Tabak AG & Jokela M. (2017) Overweight, obesity, and risk of cardiometabolic multimorbidity: pooled analysis of individual-level data for 120 813 adults from 16 cohort studies from the USA and Europe. Lancet Public Health 2(6): e277-e285.

Kjos SL, Peters RK, Xiang A, Schaefer U & Buchanan TA. (1998) Hormonal choices after gestational diabetes. Subsequent pregnancy, contraception, and hormone replacement. Diabetes Care 21 Suppl 2: B50-7.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM & Diabetes Prevention Program Research Group. (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 346(6): 393-403.

Koller A. (2002) Signaling pathways of mechanotransduction in arteriolar endothelium and smooth muscle cells in hypertension. Microcirculation 9(4): 277-294.

Kontis S & Gosling RG. (1989) On-line Doppler ultrasound measurement of aortic compliance and its repeatability in normal subjects. Clin Phys Physiol Meas 10(2): 127-135.

Kopelman PG. (2000) Obesity as a medical problem. Nature 404(6778): 635-643.

Kormi I, Nieminen MT, Havulinna AS, Zeller T, Blankenberg S, Tervahartiala T, Sorsa T, Salomaa V & Pussinen PJ. (2017) Matrix metalloproteinase-8 and tissue inhibitor of matrix metalloproteinase-1 predict incident cardiovascular disease events and all-cause mortality in a population-based cohort. Eur J Prev Cardiol 24(11): 1136-1144.

Kostapanos M, McEniery CM & Wilkinson IB. (2016) Clinical relevance of central blood pressure - a critical review. Vasa 45(6): 451-460.

Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J & Salonen JT. (2002) The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288(21): 2709-2716.

Landmesser U, Hornig B & Drexler H. (2004) Endothelial function: a critical determinant in atherosclerosis? Circulation 109(21 Suppl 1): II27-33.

Lane HA, Smith JC & Davies JS. (2006) Noninvasive assessment of preclinical atherosclerosis. Vasc Health Risk Manag 2(1): 19-30.

Lann D & LeRoith D. (2007) Insulin resistance as the underlying cause for the metabolic syndrome. Med Clin North Am 91(6): 1063-77.

Latham RD, Westerhof N, Sipkema P, Rubal BJ, Reuderink P & Murgo JP. (1985) Regional wave travel and reflections along the human aorta: a study with six simultaneous micromanometric pressures. Circulation 72(6): 1257-1269.

Lauenborg J, Grarup N, Damm P, Borch-Johnsen K, Jorgensen T, Pedersen O & Hansen T. (2009) Common type 2 diabetes risk gene variants associate with gestational diabetes. J Clin Endocrinol Metab 94(1): 145-150.

Lauenborg J, Mathiesen E, Hansen T, Glumer C, Jorgensen T, Borch-Johnsen K, Hornnes P, Pedersen O & Damm P. (2005) The prevalence of the metabolic syndrome in a danish population of women with previous gestational diabetes mellitus is three-fold higher than in the general population. J Clin Endocrinol Metab 90(7): 4004-4010.

Lauhio A, Färkkilä E, Pietiläinen KH, Åström P, Winkelmann A, Tervahartiala T, Pirilä E, Rissanen A, Kaprio J, Sorsa TA & Salo T. (2016) Association of MMP-8 with obesity, smoking and insulin resistance. Eur J Clin Invest 46(9): 757-765.

Lauhio A, Hastbäcka J, Pettilä V, Tervahartiala T, Karlsson S, Varpula T, Varpula M, Ruokonen E, Sorsa T & Kolho E. (2011) Serum MMP-8, -9 and TIMP-1 in sepsis: high serum levels of MMP-8 and TIMP-1 are associated with fatal outcome in a multicentre, prospective cohort study. Hypothetical impact of tetracyclines. Pharmacol Res 64(6): 590-594.

Lauhio A, Konttinen YT, Tschesche H, Nordström D, Salo T, Lähdevirta J, Golub LM & Sorsa T. (1994) Reduction of matrix metalloproteinase 8-neutrophil collagenase levels during long-term doxycycline treatment of reactive arthritis. Antimicrob Agents Chemother 38(2): 400-402.

Lauhio A, Saikku P, Salo T, Tschesche H, Lähdevirta J & Sorsa T. (2011) Combination treatment in Chlamydia-triggered reactive arthritis: comment on the article by Carter et al. Arthritis Rheum 63(1): 305-7; author reply 307-8.

Lauhio A, Salo T, Ding Y, Konttinen YT, Nordström D, Tschesche H, Lähdevirta J, Golub LM & Sorsa T. (1994) In vivo inhibition of human neutrophil collagenase (MMP-8) activity during long-term combination therapy of doxycycline and non-steroidal anti-inflammatory drugs (NSAID) in acute reactive arthritis. Clin Exp Immunol 98(1): 21-28.

Lauhio A, Salo T, Tjäderhane L, Lähdevirta J, Golub LM & Sorsa T. (1995) Tetracyclines in treatment of rheumatoid arthritis. Lancet 346(8975): 645-646.

Laurent S, Boutouyrie P & Lacolley P. (2005) Structural and genetic bases of arterial stiffness. Hypertension 45(6): 1050-1055.

Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H & European Network for Non-invasive Investigation of Large Arteries. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 27(21): 2588-2605.

Lawrence JM, Contreras R, Chen W & Sacks DA. (2008) Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999-2005. Diabetes Care 31(5): 899-904.

Lee CJ & Park S. (2014) The role of carotid ultrasound for cardiovascular risk stratification beyond traditional risk factors. Yonsei Med J 55(3): 551-557.

Lee SK, Khambhati J, Varghese T, Stahl EP, Kumar S, Sandesara PB, Wenger NK & Sperling LS. (2017) Comprehensive primary prevention of cardiovascular disease in women. Clin Cardiol .

Lee YS, Lan Tran HT & Van Ta Q. (2009) Regulation of expression of matrix metalloproteinase-9 by JNK in Raw 264.7 cells: presence of inhibitory factor(s) suppressing MMP-9 induction in serum and conditioned media. Exp Mol Med 41(4): 259-268.

Lefferts WK, Sperry SD, Jorgensen RS, Kasprowicz AG, Skilton MR, Figueroa A & Heffernan KS. (2017) Carotid stiffness, extra-media thickness and visceral adiposity in young adults. Atherosclerosis 265: 140-146.

Lekva T, Bollerslev J, Norwitz ER, Aukrust P & Henriksen T. (2015) Aortic Stiffness and Cardiovascular Risk in Women with Previous Gestational Diabetes Mellitus. 10(8): e0136892.

Lekva T, Michelsen AE, Aukrust P, Henriksen T, Bollerslev J & Ueland T. (2017) Leptin and adiponectin as predictors of cardiovascular risk after gestational diabetes mellitus. Cardiovasc Diabetol 16(1): 5.

Lekva T, Michelsen AE, Bollerslev J, Norwitz ER, Aukrust P, Henriksen T & Ueland T. (2016) Low circulating pentraxin 3 levels in pregnancy is associated with gestational diabetes and increased apoB/apoA ratio: a 5-year follow-up study. Cardiovasc Diabetol 15: 23.

Lenglet S, Mach F & Montecucco F. (2013) Role of matrix metalloproteinase-8 in atherosclerosis. Mediators Inflamm 2013: 659282.

Li W, Liu H, Qiao Y, Lv F, Zhang S, Wang L, Leng J, Liu H, Qi L, Tuomilehto J & Hu G. (2015) Metabolic syndrome of weight change from pre-pregnancy to 1-5 years post-partum among Chinese women with prior gestational diabetes. Diabet Med 32(11): 1492-1499.

Lim S & Meigs JB. (2013) Ectopic fat and cardiometabolic and vascular risk. Int J Cardiol 169(3): 166-176.

Lind JM, Hennessy A & McLean M. (2014) Cardiovascular disease in women: the significance of hypertension and gestational diabetes during pregnancy. Curr Opin Cardiol 29(5): 447-453.

Lindström J, Louheranta A, Mannelin M, Rastas M, Salminen V, Eriksson J, Uusitupa M, Tuomilehto J & Finnish Diabetes Prevention Study Group. (2003) The Finnish Diabetes Prevention Study (DPS): Lifestyle intervention and 3-year results on diet and physical activity. Diabetes Care 26(12): 3230-3236.

Lindström J, Peltonen M, Eriksson JG, Ilanne-Parikka P, Aunola S, Keinänen-Kiukaanniemi S, Uusitupa M, Tuomilehto J & Finnish Diabetes Prevention Study (DPS). (2013) Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). Diabetologia 56(2): 284-293.

Lipscombe LL & Hux JE. (2007) Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995-2005: a population-based study. Lancet 369(9563): 750-756.

Liu H, Zhang C, Zhang S, Wang L, Leng J, Liu D, Fang H, Li W, Yu Z, Yang X, Dong L & Hu G. (2014) Prepregnancy body mass index and weight change on postpartum diabetes risk among gestational diabetes women. Obesity (Silver Spring) 22(6): 1560-1567.

Lloyd-Jones DM, Larson MG, Beiser A & Levy D. (1999) Lifetime risk of developing coronary heart disease. Lancet 353(9147): 89-92.

London GM & Drueke TB. (1997) Atherosclerosis and arteriosclerosis in chronic renal failure. Kidney Int 51(6): 1678-1695.

Loukogeorgakis S, Dawson R, Phillips N, Martyn CN & Greenwald SE. (2002) Validation of a device to measure arterial pulse wave velocity by a photoplethysmographic method. Physiol Meas 23(3): 581-596.

Lubos E, Schnabel R, Rupprecht HJ, Bickel C, Messow CM, Prigge S, Cambien F, Tiret L, Munzel T & Blankenberg S. (2006) Prognostic value of tissue inhibitor of metalloproteinase-1 for cardiovascular death among patients with cardiovascular disease: results from the AtheroGene study. Eur Heart J 27(2): 150-156.

Lucero D, Lopez GI, Gorzalczany S, Duarte M, Gonzalez Ballerga E, Sorda J, Schreier L & Zago V. (2016) Alterations in triglyceride rich lipoproteins are related to endothelial dysfunction in metabolic syndrome. Clin Biochem 49(12): 932-935.

Mai C, Wang B, Wen J, Lin X & Niu J. (2014) Lipoprotein-associated phospholipase A2 and AGEs are associated with cardiovascular risk factors in women with history of gestational diabetes mellitus. Gynecol Endocrinol 30(3): 241-244.

Manning AK, Hivert MF, Scott RA, Grimsby JL, Bouatia-Naji N, Chen H, Rybin D, Liu CT, Bielak LF, Prokopenko I, Amin N, Barnes D, Cadby G, Hottenga JJ, Ingelsson E, Jackson AU, Johnson T, Kanoni S, Ladenvall C, Lagou V, Lahti J, Lecoeur C, Liu Y, Martinez-Larrad MT, Montasser ME, Navarro P, Perry JR, Rasmussen-Torvik LJ, Salo P, Sattar N, Shungin D, Strawbridge RJ, Tanaka T, van Duijn CM, An P, de Andrade M, Andrews JS, Aspelund T, Atalay M, Aulchenko Y, Balkau B, Bandinelli S, Beckmann JS, Beilby JP, Bellis C, Bergman RN, Blangero J, Boban M, Boehnke M, Boerwinkle E, Bonnycastle LL, Boomsma DI, Borecki IB, Bottcher Y, Bouchard C, Brunner E, Budimir D, Campbell H, Carlson O, Chines PS, Clarke R, Collins FS, Corbaton-Anchuelo A, Couper D, de Faire U, Dedoussis GV, Deloukas P, Dimitriou M, Egan JM, Eiriksdottir G, Erdos MR, Eriksson JG, Eury E, Ferrucci L, Ford I, Forouhi NG, Fox CS, Franzosi MG, Franks PW, Frayling TM, Froguel P, Galan P, de Geus E, Gigante B, Glazer NL, Goel A, Groop L, Gudnason V, Hallmans G, Hamsten A, Hansson O, Harris TB, Hayward C, Heath S, Hercberg S, Hicks AA, Hingorani A, Hofman A, Hui J, Hung J, Jarvelin MR, Jhun MA, Johnson PC, Jukema JW, Jula A, Kao WH, Kaprio J, Kardia SL, Keinanen-Kiukaanniemi S, Kivimaki M, Kolcic I, Kovacs P, Kumari M, Kuusisto J, Kyvik KO, Laakso M, Lakka T, Lannfelt L, Lathrop GM, Launer LJ, Leander K, Li G, Lind L, Lindstrom J, Lobbens S, Loos RJ, Luan J, Lyssenko V, Magi R, Magnusson PK, Marmot M, Meneton P, Mohlke KL, Mooser V, Morken MA, Miljkovic I, Narisu N, O'Connell J, Ong KK, Oostra BA, Palmer LJ, Palotie A, Pankow JS, Peden JF, Pedersen NL, Pehlic M, Peltonen L, Penninx B, Pericic M, Perola M, Perusse L, Peyser PA, Polasek O, Pramstaller PP, Province MA, Raikkonen K, Rauramaa R, Rehnberg E, Rice K, Rotter JI, Rudan I, Ruokonen A, Saaristo T, Sabater-Lleal M, Salomaa V, Savage DB, Saxena R, Schwarz P, Seedorf U, Sennblad B, Serrano-Rios M, Shuldiner AR, Sijbrands EJ, Siscovick DS, Smit JH, Small KS, Smith NL, Smith AV, Stancakova A, Stirrups K, Stumvoll M, Sun YV, Swift AJ, Tonjes A, Tuomilehto J, Trompet S, Uitterlinden AG, Uusitupa M, Vikstrom M, Vitart V, Vohl MC, Voight BF, Vollenweider P, Waeber G, Waterworth DM, Watkins H, Wheeler E, Widen E, Wild SH, Willems SM, Willemsen G, Wilson JF, Witteman JC, Wright AF, Yaghootkar H, Zelenika D, Zemunik T, Zgaga L, DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium, Multiple Tissue Human Expression Resource (MUTHER) Consortium, Wareham NJ, McCarthy MI, Barroso I, Watanabe RM, Florez JC, Dupuis J, Meigs JB & Langenberg C. (2012) A genome-wide approach accounting for body mass index identifies genetic variants

influencing fasting glycemic traits and insulin resistance. Nat Genet 44(6): 659-669.

Mao H, Li Q & Gao S. (2012) Meta-analysis of the relationship between common type 2 diabetes risk gene variants with gestational diabetes mellitus. PLoS One 7(9): e45882.

Martin-Timon I, Sevillano-Collantes C, Segura-Galindo A & Del Canizo-Gomez FJ. (2014) Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? World J Diabetes 5(4): 444-470.

Maseri A, L'Abbate A, Baroldi G, Chierchia S, Marzilli M, Ballestra AM, Severi S, Parodi O, Biagini A, Distante A & Pesola A. (1978) Coronary vasospasm as a possible cause of myocardial infarction. A conclusion derived from the study of "preinfarction" angina. N Engl J Med 299(23): 1271-1277.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7): 412-419.

Matthews TJ & Hamilton BE. (2014) First births to older women continue to rise. NCHS Data Brief (152): 1-8.

McVeigh GE. (2003) Pulse waveform analysis and arterial wall properties. Hypertension 41(5): 1010-1011.

McVeigh GE, Bratteli CW, Morgan DJ, Alinder CM, Glasser SP, Finkelstein SM & Cohn JN. (1999) Age-related abnormalities in arterial compliance identified by pressure pulse contour analysis: aging and arterial compliance. Hypertension 33(6): 1392-1398.

McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW & Hayes JR. (1992) Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 35(8): 771-776.

McVeigh GE, Hamilton PK & Morgan DR. (2002) Evaluation of mechanical arterial properties: clinical, experimental and therapeutic aspects. Clin Sci (Lond) 102(1): 51-67.

Meigs JB, Hu FB, Rifai Nader & Manson JoAnn E. (2004) Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 291(16): 1978-1986.

Mente A, Yusuf S, Islam S, McQueen MJ, Tanomsup S, Onen CL, Rangarajan S, Gerstein HC, Anand SS & INTERHEART Investigators. (2010) Metabolic syndrome and risk of acute myocardial infarction a case-control study of 26,903 subjects from 52 countries. J Am Coll Cardiol 55(21): 2390-2398.

Mersich B, Rigo J,Jr, Besenyei C, Lenard Z, Studinger P & Kollai M. (2005) Opposite changes in carotid versus aortic stiffness during healthy human pregnancy. Clin Sci (Lond) 109(1): 103-107.

Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, Hadden DR, Hod M, Kitzmiller JL, Kjos SL, Oats JN, Pettitt DJ, Sacks DA & Zoupas C. (2007) Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care 30 Suppl 2: S251-60.

Meyers MR & Gokce N. (2007) Endothelial dysfunction in obesity: etiological role in atherosclerosis. Curr Opin Endocrinol Diabetes Obes 14(5): 365-369.

Mikael LR, Paiva AMG, Gomes MM, Sousa ALL, Jardim PCBV, Vitorino PVO, Euzebio MB, Sousa WM & Barroso WKS. (2017) Vascular Aging and Arterial Stiffness. Arq Bras Cardiol 109(3): 253-258.

Mikolasevic I, Zutelija M, Mavrinac V & Orlic L. (2017) Dyslipidemia in patients with chronic kidney disease: etiology and management. Int J Nephrol Renovasc Dis 10: 35-45.

Miller HC. (1946) The effect of diabetic and prediabetic pregnancies on the fetus and newborn infant. J Pediatr 29(4): 455-461.

Miller EL & Mitchell A. (2006) Metabolic syndrome: screening, diagnosis, and management. J Midwifery Womens Health 51(3): 141-151.

Mishra S, Rao CR & Shetty A. (2016) Trends in the Diagnosis of Gestational Diabetes Mellitus. Scientifica (Cairo) 2016: 5489015.

Mitchell GF. (2004) Increased aortic stiffness: an unfavorable cardiorenal connection. Hypertension 43(2): 151-153.

Mitsuhashi T, Hibi K, Kosuge M, Morita S, Komura N, Kusama I, Otsuka F, Endo M, Iwahashi N, Okuda J, Tsukahara K, Ebina T, Umemura S & Kimura K. (2011) Relation between hyperinsulinemia and nonculprit plaque characteristics in nondiabetic patients with acute coronary syndromes. JACC Cardiovasc Imaging 4(4): 392-401.

Miyashita H. (2012) Clinical Assessment of Central Blood Pressure. Curr Hypertens Rev 8(2): 80-90.

Mohiaddin RH, Firmin DN & Longmore DB. (1993) Age-related changes of human aortic flow wave velocity measured noninvasively by magnetic resonance imaging. J Appl Physiol (1985) 74(1): 492-497.

Monhart V. (2011) Microalbuminuria. From diabetes to cardiovascular risk. Vnitr Lek 57(3): 293-298.

Monzillo LU & Hamdy O. (2003) Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev 61(12): 397-412.

Moore CS & Crocker SJ. (2012) An alternate perspective on the roles of TIMPs and MMPs in pathology. Am J Pathol 180(1): 12-16.

Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC,Jr, Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V & Wenger NK. (2011) Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. Circulation 123(11): 1243-1262.

Mosca L, Linfante AH, Benjamin EJ, Berra K, Hayes SN, Walsh BW, Fabunmi RP, Kwan J, Mills T & Simpson SL. (2005) National study of physician awareness and adherence to cardiovascular disease prevention guidelines. Circulation 111(4): 499-510.

Mosca L, Mochari-Greenberger H, Dolor RJ, Newby LK & Robb KJ. (2010) Twelve-year follow-up of American women's awareness of cardiovascular disease risk and barriers to heart health. Circ Cardiovasc Qual Outcomes 3(2): 120-127.

Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL & Eisenberg MJ. (2010) The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol 56(14): 1113-1132.

Muntoni S, Muntoni S & Draznin B. (2008) Effects of chronic hyperinsulinemia in insulin-resistant patients. Curr Diab Rep 8(3): 233-238.

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). (2002) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 106(25): 3143-3421.

NCD Risk Factor Collaboration (NCD-RisC). (2016) Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet 387(10026): 1377-1396.

NDDG. (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. Diabetes 28(12): 1039-1057.

Negrato CA & Gomes MB. (2013) Historical facts of screening and diagnosing diabetes in pregnancy. Diabetol Metab Syndr 5(1): 22.

Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT & Tajik J. (2010) Noninvasive Measurement of Central Vascular Pressures With Arterial Tonometry: Clinical Revival of the Pulse Pressure Waveform? Mayo Clin Proc 85(5): 460-472.

Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA, Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwari P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Merriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX, Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang X, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ & Gakidou E. (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384(9945): 766-781.

Nguyen NT, Kim E, Vu S & Phelan M. (2017) Ten-year Outcomes of a Prospective Randomized Trial of Laparoscopic Gastric Bypass Versus Laparoscopic Gastric Banding. Ann Surg : [Epub ahead of print].

Nichols WW & O'Rourke MF. (2005) McDonald's blood flow in arteries. Theoretical, Experimental and Clinical Principles. 5th ed. Oxford University Press, 2005.

Nichols WW. (2005) Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. Am J Hypertens 18(1 Pt 2): 3S-10S.

Nichols WW & McDonald DA. (1972) Wave-velocity in the proximal aorta. Med Biol Eng 10(3): 327-335.

Njajou OT, Kanaya AM, Holvoet P, Connelly S, Strotmeyer ES, Harris TB, Cummings SR, Hsueh WC & Health ABC Study. (2009) Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the Health, Aging and Body Composition Study. Diabetes Metab Res Rev 25(8): 733-739.

Njolstad I, Arnesen E & Lund-Larsen PG. (1996) Smoking, serum lipids, blood pressure, and sex differences in myocardial infarction. A 12-year follow-up of the Finnmark Study. Circulation 93(3): 450-456.

Noctor E, Crowe C, Carmody LA, Kirwan B, O'Dea A, Glynn LG, McGuire BE, O'Shea PM & Dunne FP. (2015) ATLANTIC-DIP: prevalence of metabolic syndrome and insulin resistance in women with previous gestational diabetes mellitus by International Association of Diabetes in Pregnancy Study Groups criteria. Acta Diabetol 52(1): 153-160.

O'Sullivan JB & Mahan CM. (1964) Criteria for the oral glucose tolerance test in pregnancy. Diabetes 13: 278-285.

O'Sullivan JB, Mahan CM, Charles D & Dandrow RV. (1973) Screening criteria for high-risk gestational diabetic patients. Am J Obstet Gynecol 116(7): 895-900.

Obesity (adult). Current Care Guidelines. (2013) Obesity (adult). Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim and the Finnish Association for the Study of Obesity. Helsinki: The Finnish Medical Society Duodecim, 2013 (referred November 16, 2017). Available at online: www.kaypahoito.fi.

Odegaar A, Jacobs DJ, Sanchez O, Goff DJ, Reiner A & Gross M. (2016) Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. Cardiovascular diabetology 15: 51.

Ogden CL, Carroll MD, Kit BK & Flegal KM. (2014) Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 311(8): 806-814.

Ogonowski J & Miazgowski T. (2009) The prevalence of 6 weeks postpartum abnormal glucose tolerance in Caucasian women with gestational diabetes. Diabetes Res Clin Pract 84(3): 239-244.

Ohashi R, Mu H, Wang X, Yao Q & Chen C. (2005) Reverse cholesterol transport and cholesterol efflux in atherosclerosis. QJM 98(12): 845-856.

Ohnishi H, Saitoh S, Akasaka H, Furukawa T, Mori M & Miura T. (2016) Impact of longitudinal status change in metabolic syndrome defined by two different criteria on new onset of type 2 diabetes in a general Japanese population: the Tanno-Sobetsu Study. Diabetol Metab Syndr 8(1): 64.

Orakzai SH, Nasir K, Blaha M, Blumenthal RS & Raggi P. (2009) Non-HDL cholesterol is strongly associated with coronary artery calcification in asymptomatic individuals. Atherosclerosis 202(1): 289-295.

O'Rourke M. (1995) Mechanical principles in arterial disease. Hypertension 26(1): 2-9.

Oyama-Kato M, Ohmichi M, Takahashi K, Suzuki S, Henmi N, Yokoyama Y & Kurachi H. (2006) Change in pulse wave velocity throughout normal pregnancy and its value in predicting pregnancy-induced hypertension: a longitudinal study. Am J Obstet Gynecol 195(2): 464-469.

Ozuguz U, Isik S, Berker D, Arduc A, Tutuncu Y, Akbaba G, Gokay F & Guler S. (2011) Gestational diabetes and subclinical inflammation: evaluation of first year postpartum outcomes. Diabetes Res Clin Pract 94(3): 426-433.

Paim LR, Schreiber R, Matos-Souza JR, Silva AA, Campos LF, Azevedo ER, Alonso K, de Rossi G, Etchebehere M, Gorla JI, Cliquet A,Jr & Nadruz W,Jr. (2013) Oxidized low-density lipoprotein, matrix-metalloproteinase-8 and carotid atherosclerosis in spinal cord injured subjects. Atherosclerosis 231(2): 341-345.

Pan A, Wang Y, Talaei M & Hu FB. (2015) Relation of Smoking With Total Mortality and Cardiovascular Events Among Patients With Diabetes Mellitus: A Meta-Analysis and Systematic Review. Circulation 132(19): 1795-1804.

Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH & Howard BV. (1997) Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 20(4): 537-544.

Pandey AK, Pandey S, Blaha MJ, Agatston A, Feldman T, Ozner M, Santos RD, Budoff MJ, Blumenthal RS & Nasir K. (2013) Family history of coronary heart disease and markers of subclinical cardiovascular disease: where do we stand? Atherosclerosis 228(2): 285-294.

Papaioannou TG, Protogerou AD, Stamatelopoulos KS, Vavuranakis M & Stefanadis C. (2009) Non-invasive methods and techniques for central blood pressure estimation: procedures, validation, reproducibility and limitations. Curr Pharm Des 15(3): 245-253.

Parikh H, Lyssenko V & Groop LC. (2009) Prioritizing genes for follow-up from genome wide association studies using information on gene expression in tissues relevant for type 2 diabetes mellitus. BMC Med Genomics 2: 72-8794-2-72.

Park SE, Park CY & Sweeney G. (2015) Biomarkers of insulin sensitivity and insulin resistance: Past, present and future. Crit Rev Clin Lab Sci 52(4): 180-190.

Parthasarathy S, Steinberg D & Witztum JL. (1992) The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. Annu Rev Med 43: 219-225.

Pearce N, Ebrahim S, McKee M, Lamptey P, Barreto ML, Matheson D, Walls H, Foliaki S, Miranda J, Chimeddamba O, Marcos LG, Haines A & Vineis P. (2014) The road to 25x25: how can the five-target strategy reach its goal? Lancet Glob Health 2(3): e126-8.

Peltonen M, Harald K, Männistö S, Saarikoski L, Lund L, Sundvall J, Juolevi A, Laatikainen T, Aldén-Nieminen H, Luoto R, Jousilahti P, Salomaa V, Taimi M & Vartiainen E. (2008) Kansallinen FINRISKI 2007 -terveystutkimus - Tutkimuksen toteutus ja tulokset: Taulukkoliite. Kansanterveyslaitoksen julkaisuja B35/2008. Helsinki 2008.

Peluso I, Morabito G, Urban L, Ioannone F & Serafini M. (2012) Oxidative stress in atherosclerosis development: the central role of LDL and oxidative burst. Endocr Metab Immune Disord Drug Targets 12(4): 351-360.

Pepys MB & Hirschfield GM. (2003) C-reactive protein: a critical update. J Clin Invest 111(12): 1805-1812.

Pickup JC. (2004) Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 27(3): 813-823.

Pirkola J, Pouta A, Bloigu A, Miettola S, Hartikainen AL, Järvelin MR & Vääräsmäki M. (2010) Prepregnancy overweight and gestational diabetes as determinants of subsequent diabetes and hypertension after 20-year follow-up. J Clin Endocrinol Metab 95(2): 772-778.

Pohjantähti-Maaroos H, Palomäki A, Kankkunen P, Husgafvel S, Knuth T, Vesterinen K & Oksanen K. (2012) Arterial elasticity and oxidized LDL among men with metabolic syndrome and different 10-year cardiovascular risk estimated by FINRISK and SCORE models. Ann Med 44(5): 503-512.

Poola-Kella S, Steinman RA, Mesmar B & Malek R. (2017) Gestational Diabetes Mellitus: Post-partum Risk and Follow up. Rev Recent Clin Trials : [Epub ahead of print].

Powell KE, Thompson PD, Caspersen CJ & Kendrick JS. (1987) Physical activity and the incidence of coronary heart disease. Annu Rev Public Health 8: 253-287.

Prasad RB & Groop L. (2015) Genetics of type 2 diabetes-pitfalls and possibilities. Genes (Basel) 6(1): 87-123.

Prenner SB & Chirinos JA. (2015) Arterial stiffness in diabetes mellitus. Atherosclerosis 238(2): 370-379.

Prudente S, Morini E & Trischitta V. (2009) Insulin signaling regulating genes: effect on T2DM and cardiovascular risk. Nat Rev Endocrinol 5(12): 682-693.

Puhkala J, Kinnunen TI, Vasankari T, Kukkonen-Harjula K, Raitanen J & Luoto R. (2013) Prevalence of metabolic syndrome one year after delivery in Finnish women at increased risk for gestational diabetes mellitus during pregnancy. J Pregnancy 2013: 139049.

Pussinen PJ, Sarna S, Puolakkainen M, Ohlin H, Sorsa T & Pesonen E. (2013) The balance of serum matrix metalloproteinase-8 and its tissue inhibitor in acute coronary syndrome and its recurrence. Int J Cardiol 167(2): 362-368.

Pyörälä K. (1979) Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. Diabetes Care 2(2): 131-141.

Qin R, Chen T, Lou Q & Yu D. (2013) Excess risk of mortality and cardiovascular events associated with smoking among patients with diabetes: meta-analysis of observational prospective studies. Int J Cardiol 167(2): 342-350.

Ramachandran A, Ma RC & Snehalatha C. (2010) Diabetes in Asia. Lancet 375(9712): 408-418.

Rani PR & Begum J. (2016) Screening and Diagnosis of Gestational Diabetes Mellitus, Where Do We Stand. J Clin Diagn Res 10(4): QE01-4.

Rao Kondapally Seshasai S, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njolstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J & Emerging Risk Factors Collaboration. (2011) Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med 364(9): 829-841.

Rautelin HI, Oksanen AM, Veijola LI, Sipponen PI, Tervahartiala TI, Sorsa TA & Lauhio A. (2009) Enhanced systemic matrix metalloproteinase response in Helicobacter pylori gastritis. Ann Med 41(3): 208-215.

Reaven G. (1988) Role of insulin resistance in human disease. Diabetes 37: 595-607.

Reaven GM. (1992) Syndrome X. Blood Press Suppl 4: 13-16.

Reinblatt SL, Morin L & Meltzer SJ. (2006) The importance of a postpartum 75 g oral glucose tolerance test in women with gestational diabetes. J Obstet Gynaecol Can 28(8): 690-694.

Report of a WHO consultation. (2000) Obesity: preventing and managing the global epidemic. World Health Organ Tech Rep Ser 894: i-xii, 1-253.

Report of a WHO Expert Committee. (1995) Physical status: the use and interpretation of anthropometry. World Health Organ Tech Rep Ser 854: 1-452.

Retnakaran R, Qi Y, Connelly PW, Sermer M, Zinman B & Hanley AJ. (2010) Glucose intolerance in pregnancy and postpartum risk of metabolic syndrome in young women. J Clin Endocrinol Metab 95(2): 670-677.

Retnakaran R, Qi Y, Sermer M, Connelly PW, Zinman B & Hanley AJ. (2009) Comparison of National Diabetes Data Group and American Diabetes Association diagnostic criteria for gestational diabetes in their identification of postpartum risk of glucose intolerance. Diabetes Res Clin Pract 85(1): 40-46.

Retnakaran R & Shah BR. (2017) Role of Type 2 Diabetes in Determining Retinal, Renal, and Cardiovascular Outcomes in Women With Previous Gestational Diabetes Mellitus. Diabetes Care 40(1): 101-108.

Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM,Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ & JUPITER Study Group. (2008) Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 359(21): 2195-2207.

Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM,Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, Macfadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ & JUPITER Trial Study Group. (2009) Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. Lancet 373(9670): 1175-1182.

Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ & CANTOS Trial Group. (2017) Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. Lancet : [Epub ahead of print].

Ridker PM, Rifai N, Rose L, Buring JE & Cook NR. (2002) Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 347(20): 1557-1565.

Rocha VZ & Libby P. (2009) Obesity, inflammation, and atherosclerosis. Nat Rev Cardiol 6(6): 399-409.

Ross R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362(6423): 801-809.

Sanchez A, Mirabel JL, Barrenechea E, Eugui J, Puelles A & Castaneda A. (2002) Evaluation of an improved immunoturbidimetic assay for serum C-reactive protein on a COBAS INTEGRA 400 Analyzer. Clin Lab 48(5-6): 313-317.

Sanin V, Pfetsch V & Koenig W. (2017) Dyslipidemias and Cardiovascular Prevention: Tailoring Treatment According to Lipid Phenotype. Curr Cardiol Rep 19(7): 61.

Santilli F, D'Ardes D, Guagnano MT & Davi G. (2017) Metabolic Syndrome: Sex-Related Cardiovascular Risk and Therapeutic Approach. Curr Med Chem 24(24): 2602-2627.

Saquib,Nazmus, Stefanick M,L., Natarajan,Loki & Pierce J,P. (2013) Mortality risk in former smokers with breast cancer: Pack-years vs. smoking status. Int J Cancer 133(10): 2493-2497.

Sasson S. (2017) Nutrient overload, lipid peroxidation and pancreatic beta cell function. Free Radic Biol Med 111: 102-109.

Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM & Shepherd J. (2003) Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. Circulation 108(4): 414-419.

Saucedo R, Zarate A, Basurto L, Hernandez M, Puello E, Galvan R & Campos S. (2011) Relationship between circulating adipokines and insulin resistance during pregnancy and postpartum in women with gestational diabetes. Arch Med Res 42(4): 318-323.

Schächinger V, Britten MB & Zeiher AM. (2000) Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101(16): 1899-1906.

Schmidt L, Sobotka T, Bentzen JG, Nyboe Andersen A & ESHRE Reproduction and Society Task Force. (2012) Demographic and medical consequences of the postponement of parenthood. Hum Reprod Update 18(1): 29-43.

Schroeder AP & Falk E. (1995) Vulnerable and dangerous coronary plaques. Atherosclerosis 118 Suppl: S141-9.

Seshiah V, Balaji V, Shah SN, Joshi S, Das AK, Sahay BK, Banerjee S, Zargar AH & Balaji M. (2012) Diagnosis of gestational diabetes mellitus in the community. J Assoc Physicians India 60: 15-17.

Shah BR, Retnakaran R & Booth GL. (2008) Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. Diabetes Care 31(8): 1668-1669.

Shea AK, Shah BR, Clark HD, Malcolm J, Walker M, Karovitch A & Keely EJ. (2011) The effectiveness of implementing a reminder system into routine clinical practice: does it increase postpartum screening in women with gestational diabetes? Chronic Dis Can 31(2): 58-64.

Shephard RJ & Balady GJ. (1999) Exercise as cardiovascular therapy. Circulation 99(7): 963-972.

Shin JA, Lee JH, Lim SY, Ha HS, Kwon HS, Park YM, Lee WC, Kang MI, Yim HW, Yoon KH & Son HY. (2013) Metabolic syndrome as a predictor of type 2 diabetes, and its clinical interpretations and usefulness. J Diabetes Investig 4(4): 334-343.

Shostrom DCV, Sun Y, Oleson JJ, Snetselaar LG & Bao W. (2017) History of Gestational Diabetes Mellitus in Relation to Cardiovascular Disease and Cardiovascular Risk Factors in US Women. Front Endocrinol (Lausanne) 8: 144.

Siasos G, Tousoulis D, Kioufis S, Oikonomou E, Siasou Z, Limperi M, Papavassiliou AG & Stefanadis C. (2012) Inflammatory mechanisms in atherosclerosis: the impact of matrix metalloproteinases. Curr Top Med Chem 12(10): 1132-1148.

Sigurdardottir V, Fagerberg B & Hulthe J. (2002) Circulating oxidized low-density lipoprotein (LDL) is associated with risk factors of the metabolic syndrome and LDL size in clinically healthy 58-year-old men (AIR study). J Intern Med 252(5): 440-447.

Simmons RK, Alberti KG, Gale EA, Colagiuri S, Tuomilehto J, Qiao Q, Ramachandran A, Tajima N, Brajkovich Mirchov I, Ben-Nakhi A, Reaven G, Hama Sambo B, Mendis S & Roglic G. (2010) The metabolic syndrome: useful concept or clinical tool? Report of a WHO Expert Consultation. Diabetologia 53(4): 600-605.

Singh AS, Mulder C, Twisk JW, van Mechelen W & Chinapaw MJ. (2008) Tracking of childhood overweight into adulthood: a systematic review of the literature. Obes Rev 9(5): 474-488.

Singh RB, Mengi SA, Xu YJ, Arneja AS & Dhalla NS. (2002) Pathogenesis of atherosclerosis: A multifactorial process. Exp Clin Cardiol 7(1): 40-53.

Small DM. (1988) Mechanisms of reversed cholesterol transport. Agents Actions Suppl 26: 135-146.

Smith SC,Jr, Milani RV, Arnett DK, Crouse JR,3rd, McDermott MM, Ridker PM, Rosenson RS, Taubert KA, Wilson PW & American Heart Association. (2004) Atherosclerotic Vascular Disease Conference: Writing Group II: risk factors. Circulation 109(21): 2613-2616.

Sorsa T, Tervahartiala T, Leppilahti J, Hernandez M, Gamonal J, Tuomainen AM, Lauhio A, Pussinen PJ & Mäntylä P. (2011) Collagenase-2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. Pharmacol Res 63(2): 108-113.

Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL & Mäntylä P. (2006) Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Ann Med 38(5): 306-321.

Spencer L, Rollo M, Hauck Y, MacDonald-Wicks L, Wood L, Hutchesson M, Giglia R, Smith R & Collins C. (2015) The effect of weight management interventions that include a diet component on weight-related outcomes in pregnant and postpartum women: a systematic review protocol. JBI Database System Rev Implement Rep 13(1): 88-98.

Stafeev IS, Vorotnikov AV, Ratner EI, Menshikov MY & Parfyonova YV. (2017) Latent Inflammation and Insulin Resistance in Adipose Tissue. Int J Endocrinol 2017: 5076732.

Stamler J, Vaccaro O, Neaton JD & Wentworth D. (1993) Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 16(2): 434-444.

Steinberg D. (1988) Atherosclerosis Reviews, eds. Stokes, J., III, & Mancini, M. (Raven, New York) 1988;18:1-23.

Steinberg D. (2009) The LDL modification hypothesis of atherogenesis: an update. J Lipid Res 50 Suppl: S376-81.

Steinhart JR, Sugarman JR & Connell FA. (1997) Gestational diabetes is a herald of NIDDM in Navajo women. High rate of abnormal glucose tolerance after GDM. Diabetes Care 20(6): 943-947.

Stetler-Stevenson WG. (2008) Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. Sci Signal 1(27): re6.

Stocker R & Keaney JF,Jr. (2004) Role of oxidative modifications in atherosclerosis. Physiol Rev 84(4): 1381-1478.

Stout RW. (1990) Insulin and atheroma. 20-yr perspective. Diabetes Care 13(6): 631-654.

Takeoka A, Tayama J, Yamasaki H, Kobayashi M, Ogawa S, Saigo T, Kawano H, Abiru N, Hayashida M, Maeda T & Shirabe S. (2016) Intra-abdominal fat accumulation is a hypertension risk factor in young adulthood: A cross-sectional study. Medicine (Baltimore) 95(45): e5361.

Tam WH, Ma RC, Chan JC, Lao TT, Chan MH & Li CY. (2012) PP103. Arterial stiffness in women with previous GDM - A follow up of Chinese HAPO study cohort. Pregnancy Hypertens 2(3): 295.

Tam WH, Ma RC, Yang X, Ko GT, Lao TT, Chan MH, Lam CW, Cockram CS & Chan JC. (2012) Cardiometabolic risk in Chinese women with prior gestational diabetes: a 15-year follow-up study. Gynecol Obstet Invest 73(2): 168-176.

Tam WH, Yang XL, Chan JC, Ko GT, Tong PC, Ma RC, Cockram CS, Sahota D & Rogers MS. (2007) Progression to impaired glucose regulation, diabetes and metabolic syndrome in Chinese women with a past history of gestational diabetes. Diabetes Metab Res Rev 23(6): 485-489.

Taylor B, Cheema A & Soslowsky L. (2017) Tendon Pathology in Hypercholesterolemia and Familial Hypercholesterolemia. Curr Rheumatol Rep 19(12): 76.

Tchernof A & Despres JP. (2013) Pathophysiology of human visceral obesity: an update. Physiol Rev 93(1): 359-404.

Tedgui A & Mallat Z. (1999) Atherosclerotic plaque formation. Rev Prat 49(19): 2081-2086.

The Committee on Obstetric Practice. (2011) Committee opinion no. 504: Screening and diagnosis of gestational diabetes mellitus. Obstet Gynecol 118(3): 751-753.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. (1997) Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 20(7): 1183-1197.

Tolle M, Reshetnik A, Schuchardt M, Hohne M & van der Giet M. (2015) Arteriosclerosis and vascular calcification: causes, clinical assessment and therapy. Eur J Clin Invest 45(9): 976-985.

Tong PC, Ko GT, So WY, Chiang SC, Yang X, Kong AP, Ozaki R, Ma RC, Cockram CS, Chow CC & Chan JC. (2008) Use of anti-diabetic drugs and glycaemic control in type 2 diabetes-tThe Hong Kong Diabetes Registry. Diabetes Res Clin Pract 82(3): 346-352.

Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC & Alexander RW. (1995) Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 332(8): 481-487.

Trevisan M, Liu J, Bahsas FB & Menotti A. (1998) Syndrome X and Mortality: A Population-based Study. American Journal of Epidemiology 148: 958-66.

Tuomainen AM, Nyyssönen K, Laukkanen JA, Tervahartiala T, Tuomainen TP, Salonen JT, Sorsa T & Pussinen PJ. (2007) Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men. Arterioscler Thromb Vasc Biol 27(12): 2722-2728.

Tuomilehto H, Peltonen M, Partinen M, Lavigne G, Eriksson JG, Herder C, Aunola S, Keinänen-Kiukaanniemi S, Ilanne-Parikka P, Uusitupa M, Tuomilehto J, Lindström J & Finnish Diabetes Prevention Study Group. (2009) Sleep duration, lifestyle intervention, and incidence of type 2 diabetes in impaired glucose tolerance: The Finnish Diabetes Prevention Study. Diabetes Care 32(11): 1965-1971.

Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M & Finnish Diabetes Prevention Study Group. (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344(18): 1343-1350.

Tziomalos K, Athyros VG, Karagiannis A & Mikhailidis DP. (2010) Endothelial dysfunction in metabolic syndrome: prevalence, pathogenesis and management. Nutr Metab Cardiovasc Dis 20(2): 140-146.

Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, Yin FC & Lakatta EG. (1993) Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation 88(4 Pt 1): 1456-1462.

Valiani V, Sourdet S, Schoeller DA, Mackey DC, Bauer DC, Glynn NW, Yamada Y, Harris TB, Manini TM & Health, Aging and Body Composition Study. (2017) Surveying predictors of late-life longitudinal change in daily activity energy expenditure. PLoS One 12(10): e0186289.

Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, Kaiser DR & Thuillez C. (2002) Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. Am J Hypertens 15(5): 445-452.

van der Heijden-Spek JJ, Staessen JA, Fagard RH, Hoeks AP, Boudier HA & van Bortel LM. (2000) Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. Hypertension 35(2): 637-642.

van der Meer IM, de Maat MP, Bots ML, Breteler MM, Meijer J, Kiliaan AJ, Hofman A & Witteman JC. (2002) Inflammatory mediators and cell adhesion molecules as indicators of severity of atherosclerosis: the Rotterdam Study. Arterioscler Thromb Vasc Biol 22(5): 838-842.

van Greevenbroek MM, Schalkwijk CG & Stehouwer CD. (2013) Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. Neth J Med 71(4): 174-187.

Vane JR, Anggard EE & Botting RM. (1990) Regulatory functions of the vascular endothelium. N Engl J Med 323(1): 27-36.

Vanhala MJ, Kumpusalo EA, Pitkäjärvi TK & Takala JK. (1997) Metabolic syndrome in a middle-aged Finnish population. J Cardiovasc Risk 4(4): 291-295.

Vanhoutte PM. (1989) Endothelium and control of vascular function. State of the Art lecture. Hypertension 13(6 Pt 2): 658-667.

Varela P, Spyropoulou AC, Kalogerakis Z, Vousoura E, Moraitou M & Zervas IM. (2017) Association between gestational diabetes and perinatal depressive symptoms: evidence from a Greek cohort study. Prim Health Care Res Dev 18(5): 441-447.

Verma A, Boney CM, Tucker R & Vohr BR. (2002) Insulin resistance syndrome in women with prior history of gestational diabetes mellitus. J Clin Endocrinol Metab 87(7): 3227-3235.

Vlachopoulos C, Aznaouridis K & Stefanadis C. (2010) Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 55(13): 1318-1327.

Vogel RA. (1997) Coronary risk factors, endothelial function, and atherosclerosis: a review. Clin Cardiol 20(5): 426-432.

Vrachnis N, Augoulea A, Iliodromiti Z, Lambrinoudaki I, Sifakis S & Creatsas G. (2012) Previous gestational diabetes mellitus and markers of cardiovascular risk. Int J Endocrinol 2012: 458610.

Vuori E & Gissler M. (2014) Perinatal Statistics: Parturients, Deliveries and Newborns 2013. Statistical Report 23/2014. Helsinki: National Institute for Health and Welfare, 2014.

Vuori E & Gissler M. (2017) Perinatal statistics: parturients, deliveries and newborns 2016. Statistical report 22.6.2017. Helsinki: National Institute for Health and Welfare, 2016.

Wagenknecht LE, Langefeld CD, Scherzinger AL, Norris JM, Haffner SM, Saad MF & Bergman RN. (2003) Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. Diabetes 52(10): 2490-2496.

Wakatsuki A, Ikenoue N, Shinohara K, Watanabe K & Fukaya T. (2004) Small low-density lipoprotein particles and endothelium-dependent vasodilation in postmenopausal women. Atherosclerosis 177(2): 329-336.

Walford GA, Gustafsson S, Rybin D, Stancakova A, Chen H, Liu CT, Hong J, Jensen RA, Rice K, Morris AP, Magi R, Tonjes A, Prokopenko I, Kleber ME, Delgado G, Silbernagel G, Jackson AU, Appel EV, Grarup N, Lewis JP, Montasser ME, Landenvall C, Staiger H, Luan J, Frayling TM, Weedon MN, Xie W, Morcillo S, Martinez-Larrad MT, Biggs ML, Chen YD, Corbaton-Anchuelo A, Faerch K, Gomez-Zumaquero JM, Goodarzi MO, Kizer JR, Koistinen HA, Leong A, Lind L, Lindgren C, Machicao F, Manning AK, Martin-Nunez GM, Rojo-Martinez G, Rotter JI, Siscovick DS, Zmuda JM, Zhang Z, Serrano-Rios M, Smith U, Soriguer F, Hansen T, Jorgensen TJ, Linnenberg A, Pedersen O, Walker M, Langenberg C, Scott RA, Wareham NJ, Fritsche A, Haring HU, Stefan N, Groop L, O'Connell JR, Boehnke M, Bergman RN, Collins FS, Mohlke KL, Tuomilehto J, Marz W, Kovacs P, Stumvoll M, Psaty BM, Kuusisto J, Laakso M, Meigs JB, Dupuis J, Ingelsson E & Florez JC. (2016) Genome-Wide Association Study of the Modified Stumvoll Insulin Sensitivity Index Identifies BCL2 and FAM19A2 as Novel Insulin Sensitivity Loci. Diabetes 65(10): 3200-3211.

Waugh NR, Shyangdan D, Taylor-Phillips S, Suri G & Hall B. (2013) Screening for type 2 diabetes: a short report for the National Screening Committee. Health Technol Assess 17(35): 1-90.

Weir MR. (2007) Microalbuminuria and cardiovascular disease. Clin J Am Soc Nephrol 2(3): 581-590.

Wellen KE & Hotamisligil GS. (2003) Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 112(12): 1785-1788.

Westerbacka J & Yki-Järvinen H. (2002) Arterial stiffness and insulin resistance. Semin Vasc Med 2(2): 157-164.

WHO. (1999) World Health Organisation. Definition, Diagnosis and Classification of Diabetes Mellitus and its complications. Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva: World Health Organisation Department of Noncommunicable Disease Surveillance 1999: 1-59.

WHO. (2013a) Diagnostic Criteria and Classification of Hyperglycaemia First Detected in Pregnancy. Available online at: WHO/NMH/MND/13.2.

WHO. (2013b) Global action plan for the prevention and control of noncommunicable diseases 2013–2020. Geneva, Switzerland: World Health Organization, 2013.

WHO. (2014) Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy: a World Health Organization Guideline. Diabetes Res Clin Pract 103(3): 341-363.

WHO. (2015) World Health Statistics 2015.

Widlansky ME, Gokce N, Keaney JF,Jr & Vita JA. (2003) The clinical implications of endothelial dysfunction. J Am Coll Cardiol 42(7): 1149-1160.

Wijeyaratne CN, Waduge R, Arandara D, Arasalingam A, Sivasuriam A, Dodampahala SH & Balen AH. (2006) Metabolic and polycystic ovary syndromes in indigenous South Asian women with previous gestational diabetes mellitus. BJOG 113(10): 1182-1187.

Wilenius M, Tikkakoski AJ, Tahvanainen AM, Haring A, Koskela J, Huhtala H, Kähönen M, Kööbi T, Mustonen JT & Pörsti IH. (2016) Central wave reflection is associated with peripheral arterial resistance in addition to arterial stiffness in subjects without antihypertensive medication. BMC Cardiovasc Disord 16: 131.

Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR & Webb DJ. (1998) Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 16(12 Pt 2): 2079-2084.

Willett WC, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE & Hennekens CH. (1995) Weight, weight change, and coronary heart disease in women. Risk within the 'normal' weight range. JAMA 273(6): 461-465.

Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, Hughes AD, Thurston H, O'Rourke M, CAFE Investigators, Anglo-Scandinavian Cardiac Outcomes Trial Investigators & CAFE Steering Committee and Writing Committee. (2006) Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation 113(9): 1213-1225.

Williams IL, Wheatcroft SB, Shah AM & Kearney MT. (2002) Obesity, atherosclerosis and the vascular endothelium: mechanisms of reduced nitric oxide bioavailability in obese humans. Int J Obes Relat Metab Disord 26(6): 754-764.

Williams SB, Cusco JA, Roddy MA, Johnstone MT & Creager MA. (1996) Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 27(3): 567-574.

Wilson PW, Kauppila LI, O'Donnell CJ, Kiel DP, Hannan M, Polak JM & Cupples LA. (2001) Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. Circulation 103(11): 1529-1534.

Woodward M & Tunstall-Pedoe H. (2009) The metabolic syndrome is not a sensible tool for predicting the risk of coronary heart disease. Eur J Cardiovasc Prev Rehabil 16(2): 210-214.

World Medical Association Inc. (2009) Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Indian Med Assoc 107(6): 403-405.

Wright JS, Cruickshank JK, Kontis S, Dore C & Gosling RG. (1990) Aortic compliance measured by non-invasive Doppler ultrasound: description of a method and its reproducibility. Clin Sci (Lond) 78(5): 463-468.

Xu C, Zarins CK, Pannaraj PS, Bassiouny HS & Glagov S. (2000) Hypercholesterolemia superimposed by experimental hypertension induces differential distribution of collagen and elastin. Arterioscler Thromb Vasc Biol 20(12): 2566-2572.

Xu Y, Shen S, Sun L, Yang H, Jin B & Cao X. (2014) Metabolic syndrome risk after gestational diabetes: a systematic review and meta-analysis. PLoS One 9(1): e87863.

Yamashita H, Shimada K, Seki E, Mokuno H & Daida H. (2003) Concentrations of interleukins, interferon, and C-reactive protein in stable and unstable angina pectoris. Am J Cardiol 91(2): 133-136.

Yarnell JW, Patterson CC, Sweetnam PM, Thomas HF, Bainton D, Elwood PC, Bolton CH & Miller NE. (2001) Do total and high density lipoprotein cholesterol and triglycerides act independently in the prediction of ischemic heart disease? Ten-year follow-up of Caerphilly and Speedwell Cohorts. Arterioscler Thromb Vasc Biol 21(8): 1340-1345.

Zhang DX & Gutterman DD. (2007) Mitochondrial reactive oxygen species-mediated signaling in endothelial cells. Am J Physiol Heart Circ Physiol 292(5): H2023-31.

Zhu Y & Zhang C. (2016) Prevalence of Gestational Diabetes and Risk of Progression to Type 2 Diabetes: a Global Perspective. Curr Diab Rep 16(1): 7.

Zieman SJ, Melenovsky V & Kass DA. (2005) Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol 25(5): 932-943.

10 ORIGINAL PUBLICATIONS

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DIABETOLOGY & METABOLIC SYNDROME

RESEARCH Open Access

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

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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

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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 categorial 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

	GDM (n = 120)	Control (n = 120)	*p* value
75-g OGTT			
- 0 h, mmol/L	5.4 ± 0.5	4.7 ± 0.3	< 0.001
- 1 h, mmol/L	9.5 ± 2.3	7.1 ± 1.4	< 0.001
- 2 h, mmol/L	7.7 ± 2.0	5.8 ± 1.0	< 0.001
Pregnancy disorders			
- Gestational hypertension, n (%)	12 (10%)	6 (5%)	NS
- Pre-eclampsia, n (%)	7 (5.8%)	2 (1.7%)	NS
- Glucosuria, n (%)	25 (20.8%)	4 (3.3%)	< 0.001
- Proteinuria, n (%)	19 (15.8%)	7 (5.8%)	0.021
Induction of delivery, n (%)	42 (35.0%)	26 (21.7%)	0.031
Caesarean section, n (%)	29 (24.2%)	21 (17.5%)	NS
Perinatal outcome			
- Gestational age, days	277.1 ± 9.5	278.8 ± 10.4	NS
- Birth weight of the child, g	3633 ± 519	3540 ± 471	NS
- Apgar score at one minute	8.6 ± 1.2	8.7 ± 1.4	NS
- Apgar score at five minute	9.3 ± 0.8	9.3 ± 0.8	NS
- Umbilical blood arterial pH	7.29 ± 0.1	7.28 ± 0.1	NS (0.054)
- Umbilical blood venous pH	7.35 ± 0.1	7.35 ± 0.1	NS

Data are presented as mean ± SD if not mentioned otherwise.
OGTT: Oral glucose tolerance test.

Table 2 Clinical characteristics in the GDM and control groups

	GDM (n = 120)	Control (n = 120)	*p* value
Age at follow-up, years	35.8 ± 4.4	35.9 ± 4.6	NS
Family history of			
- Coronary heart disease, n (%)	20 (16.7%)	23 (19.2%)	NS
- Cerebrovascular disease, n (%)	15 (12.5%)	5 (4.2%)	0.033
- Diabetes mellitus, n (%)	32 (26.7%)	27 (22.5%)	NS
Diagnosed disorder, n (%)	52 (43.3%)	45 (37.5%)	NS
- Hypertension, n (%)	3 (2.5%)	5 (4.2%)	NS
- Type 1 diabetes mellitus, n (%)	2 (1.7%)	0 (0%)	NS
- Type 2 diabetes mellitus, n (%)	1 (0.8%)	0 (0%)	NS
- Polycystic ovary syndrome, n (%)	8 (6.7%)	1 (0.8%)	0.036
Permanent medication for any chronic disease, n (%)	43 (35.8%)	35 (29.2%)	NS
Contraception, n (%)	99 (82.5%)	92 (76.7%)	NS
Smoking status			0.018
- Current, n (%)	24 (20.0%)	12 (10%)	
- Former, n (%)	45 (37.5%)	37 (30.8%)	
- Never, n (%)	51 (42.5%)	71 (59.2%)	
BMI, kg/m2	28.3 ± 5.0	27.5 ± 5.4	NS (0.069)
Waist circumference, cm	96.8 ± 13.0	92.5 ± 12.6	0.009
Systolic blood pressure, mmHg	122.4 ± 12.5	119.0 ± 11.5	0.034
Diastolic blood pressure, mmHg	73.5 ± 9.0	71.8 ± 8.7	NS
Heart rate, beats per minute	65.9 ± 9.1	63.8 ± 9.6	0.017
MetS, n (%)	19 (15.8%)	8 (6.7%)	0.039
- Waist circumference > 88 cm, n (%)	89 (74.2%)	73 (60.8%)	0.038
- Blood pressure ≥ 130/85 mmHg, n (%)	35 (29.2%)	25 (20.8%)	NS
- HDL cholesterol < 1.30 mmol/L, n (%)	23 (19.2%)	22 (18.3%)	NS
- Triglycerides ≥ 1.7 mmol/L, n (%)	12 (10.0%)	5 (4.2%)	NS (0.084)
- Glucose ≥ 6.1 mmol/L or diabetes, n (%)	18 (15.0%)	4 (3.3%)	0.002

Data are presented as mean ± SD if not mentioned otherwise. 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/m^2). 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.

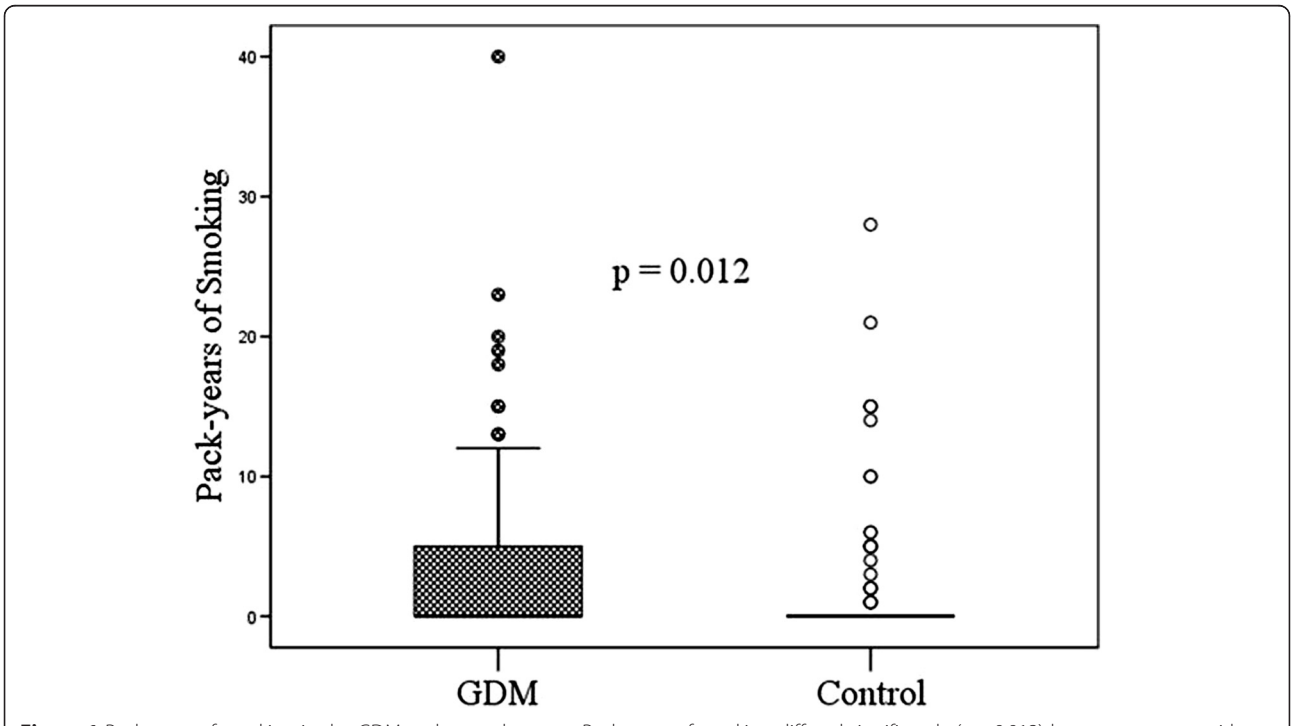

Figure 1 Pack-years of smoking in the GDM and control groups. Pack-years of smoking differed significantly (p = 0.012) between women with a previous history of GDM vs. women unaffected. The median in both groups was zero, because the majority were non-smokers. The mean (±SD) number of pack-years in the GDM group was 3.1 (±6.1) and in the control group, 1.6 (±4.4).

Table 3 Laboratory characteristics of participants with GDM vs. controls

	GDM (n = 119)	Control (n = 120)	*p* value
Leucocytes, 109/L	5.8 ± 1.6	5.2 ± 1.4	0.008
Haemoglobin, g/L	133.2 ± 9.3	128.6 ± 12.9	0.001
Platelets, 109/L	241.9 ± 58.2	244.0 ± 52.5	NS
ALAT, U/L	22.8 ± 17.4	19.7 ± 10.5	NS
Creatinine, umol/L	66.6 ± 7.7	64.5 ± 7.8	0.048
U-AlbCre, mg/mmol	0.67 ± 0.5	0.57 ± 0.3	NS (0.070)
Fibrinogen, g/L	3.4 ± 0.9	3.2 ± 1.0	NS (0.096)
Fasting glucose, mmol/L	5.6 ± 0.6	5.3 ± 0.3	< 0.001
Total cholesterol, mmol/L	4.7 ± 0.9	4.6 ± 0.8	NS
HDL cholesterol, mmol/L	1.5 ± 0.3	1.6 ± 0.3	NS
LDL cholesterol, mmol/L	3.0 ± 0.7	2.8 ± 0.6	NS
Triglycerides, mmol/L	1.1 ± 0.6	0.9 ± 0.4	< 0.001

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

The primary outcome was to define the prevalence of MetS and its different variables in the GDM and control groups. We also 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.

Statistical analyses

Statistics were analysed 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. Categorial data are presented as percentages and were compared by using the chi-square test. All 240 women were also included in subgroup analyses to study the effect of excess body weight on the results. For these analyses, we divided the whole study group into two halves according to BMI, using a cut-off point of 27 kg/m^2. According to the FINRISK 2012 Study our BMI cut-off of 27 kg/m^2

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

	GDM cases		Controls		Overall
	BMI ≥ 27 (n = 65)	BMI < 27 (n = 55)	BMI ≥ 27 (n = 57)	BMI < 27 (n = 63)	*p* value
Systolic blood pressure, mmHg*	126.6 ± 12.3	117.7 ± 11.2	122.8 ± 12.4	116.1 ± 9.1	< 0.001
Diastolic blood pressure, mmHg*	76.1 ± 9.6	70.5 ± 9.6	74.6 ± 8.1	69.1 ± 8.5	< 0.001
Mean peripheral pressure, mmHg*	94.0 ± 10.7	87.0 ± 8.4	91.5 ± 9.3	85.3 ± 8.8	< 0.001
Heart rate, beats per minute	66.6 ± 8.9	65.2 ± 9.3	65.2 ± 9.0	62.6 ± 10.1	NS
MetS, n (%)	15 (23.1 %)	4 (3.3 %)	8 (14.0 %)	0 (0 %)	< 0.001
- Waist circumference > 88 cm, n (%)	62 (95.4 %)	27 (49.1 %)	53 (93.0 %)	20 (31.7 %)	< 0.001
- Blood pressure ≥ 130/85 mmHg, n (%)	27 (41.5 %)	8 (14.5 %)	19 (33.3 %)	6 (9.5 %)	< 0.001
- HDL cholesterol < 1.30 mmol/L, n (%)	14 (21.5 %)	9 (16.4 %)	14 (24.6 %)	8 (12.7 %)	NS
- Triglycerides ≥ 1.7 mmol/L, n (%)	9 (13.8 %)	3 (5.5 %)	4 (7.0 %)	1 (1.6 %)	NS (0.050)
- Glucose ≥ 6.1 mmol/L or diabetes, n (%)	11 (16.9 %)	7 (12.7%)	1 (1.8 %)	3 (4.8 %)	0.012

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.

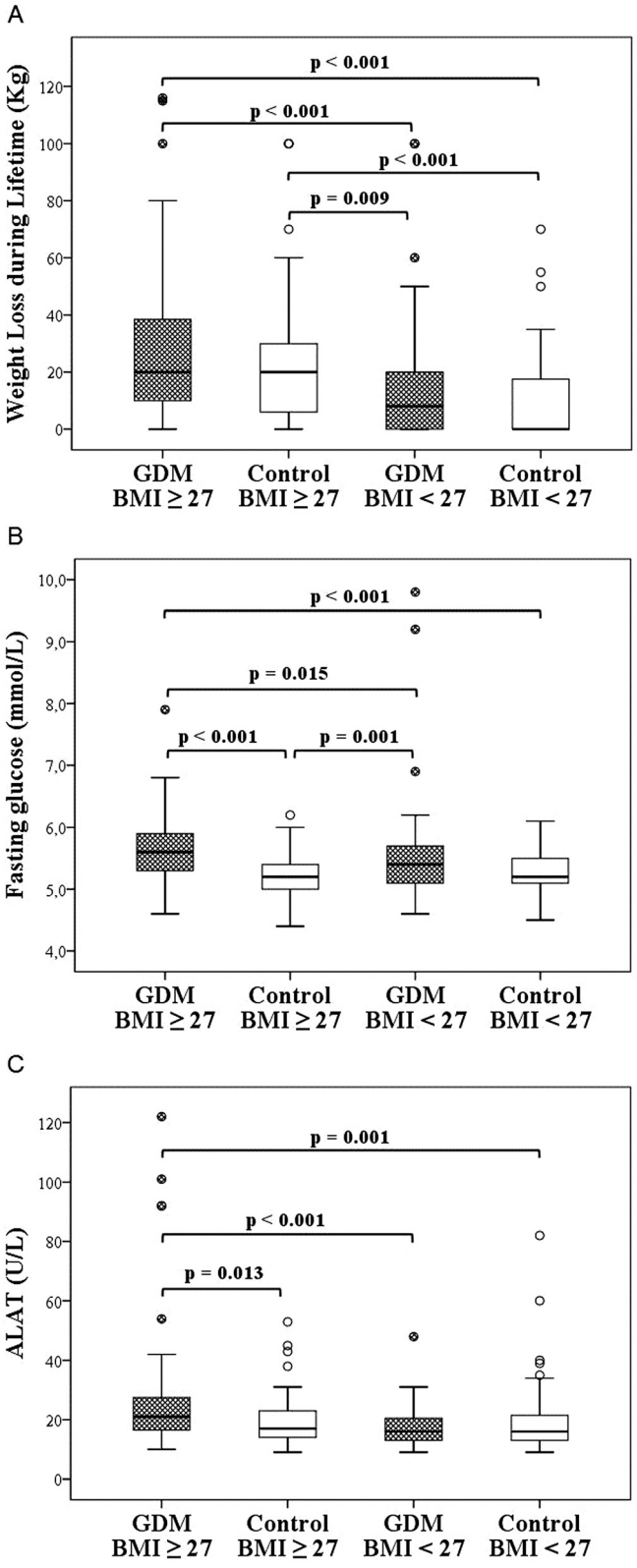

Figure 2 (See legend on next page.)

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Figure 2 Lifetime weight loss, fasting glucose and alanine transaminase in the subgroups. **A**: Median (minimum, maximum) lifetime weight loss among obese (BMI ≥ 27) GDM women was 20 (0, 116) kg, among obese control women 20 (0, 100) kg, among non-obese GDM women 8 (0, 100) kg and among non-obese control women 0 (0, 70) kg. **B**: Median (minimum, maximum) fasting glucose levels among obese GDM women 5.6 (4.6, 7.9) mmol/L, among obese control women 5.2 (4.4, 6.2) mmol/L, among non-obese GDM women 5.4 (4.6, 9.8) mmol/L and among non-obese control women 5.2 (4.5, 6.1) mmol/L. **C**: Median (minimum, maximum) alanine transaminase levels among obese GDM women 21 (10, 122) U/L, among obese control women 17 (9, 53) U/L, among non-obese GDM women 16 (9, 48) U/L and among non-obese control women 16 (9, 82).

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/m^2 [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 did 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

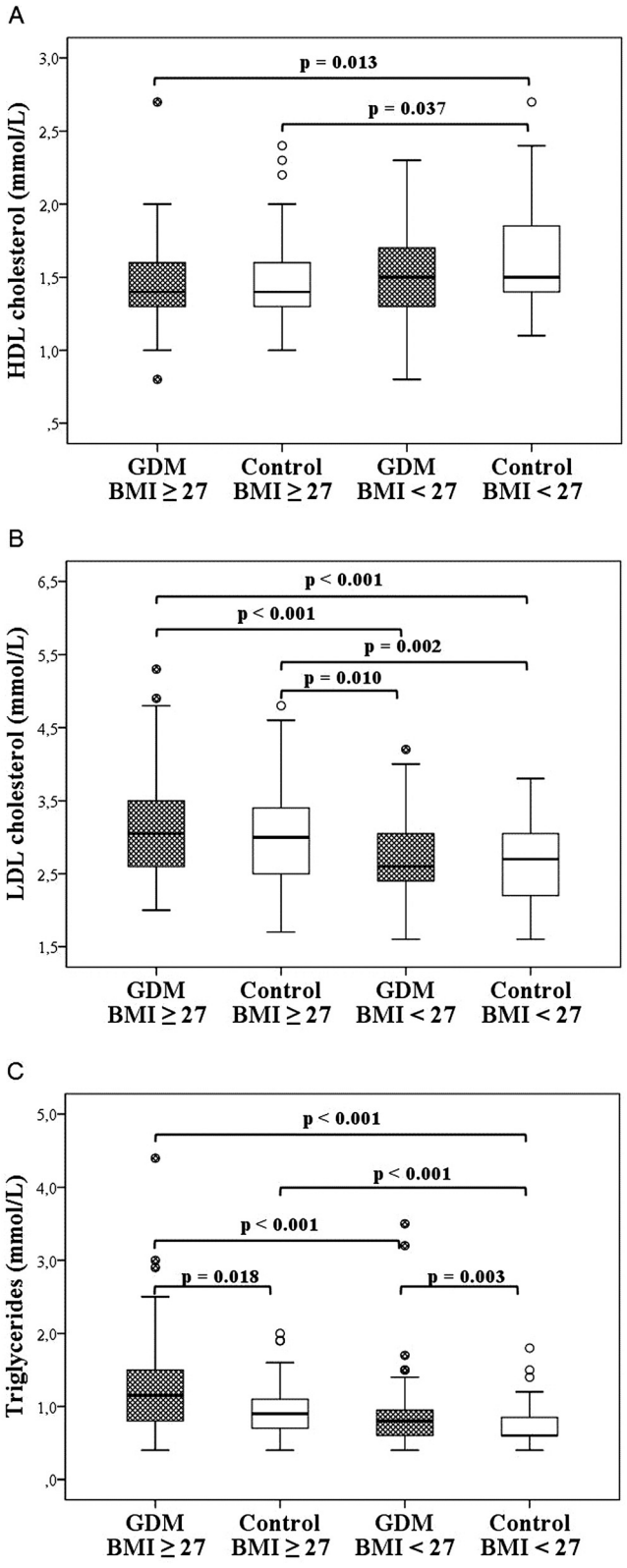

Figure 3 (See legend on next page.)

(See figure on previous page.)
Figure 3 HDL cholesterol, LDL cholesterol and triglyceride levels in the subgroups. **A**: The median (minimum, maximum) HDL cholesterol level among obese (BMI ≥ 27) GDM women was 1.4 (0.8, 2.7) mmol/L, among obese control women 1.4 (1.0, 2.4) mmol/L, among non-obese GDM women 1.5 (0.8, 2.3) mmol/L and among non-obese control women 1.5 (1.1, 2.7) mmol/L. **B**: The median (minimum, maximum) LDL cholesterol level among obese GDM women was 3.1 (2.0, 5.3) mmol/L, among obese control women 3.0 (1.7, 4.8) mmol/L, among non-obese GDM women 2.6 (1.6, 4.2) mmol/L and among non-obese control women 2.7 (1.6, 3.8) mmol/L. **C**: The median (minimum, maximum) triglyceride level among obese GDM women was 1.2 (0.4, 4.4) mmol/L, among obese control women 0.9 (0.5, 2.0) mmol/L, among non-obese GDM women 0.8 (0.4, 3.5) mmol/L and among non-obese control women 0.6 (0.4, 1.8) mmol/L.

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 1.98, 95% CI 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/m^2, and no family history of DM, or multiparous women < 40 years old, no GDM in previous pregnancy or pregnancies, and BMI ≤ 25 kg/m^2 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/m^2, because obesity is commonly classified as BMI of ≥ 30 kg/m^2 [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/m^2 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/m^2. 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

1. Vuori E, Gissler M. Perinatal statistics: parturients, deliveries and newborns 2013 statistical report 23/2014. Helsinki: National Institute for Health and Welfare; 2014.
2. Dabelea D, Snell-Bergeon JK, Hartsfield CL, Bischoff KJ, Hamman RF, McDuffie RS. Kaiser Permanente of Colorado GDM screening program: increasing prevalence of gestational diabetes mellitus (GDM) over time and by birth cohort: Kaiser Permanente of Colorado GDM screening program. Diabetes Care. 2005;28(3):579–84.
3. Järvelä IY, Juutinen J, Koskela P, Hartikainen AL, Kulmala P, Knip M, et al. Gestational diabetes identifies women at risk for permanent type 1 and type 2 diabetes in fertile age: predictive role of autoantibodies. Diabetes Care. 2006;29(3):607–12.
4. Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes care. 2002;25(10):1862–8.
5. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet. 2009;373(9677):1773–9.
6. Xu Y, Shen S, Sun L, Yang H, Jin B, Cao X. Metabolic syndrome risk after gestational diabetes: a systematic review and meta-analysis. PLoS One. 2014;9(1):e87863.
7. Lauenborg J, Mathiesen E, Hansen T, Glumer C, Jorgensen T, Borch-Johnsen K, et al. The prevalence of the metabolic syndrome in a danish population of women with previous gestational diabetes mellitus is three-fold higher than in the general population. J Clin Endocrinol Metab. 2005;90(7):4004–10.
8. Vrachnis N, Augoulea A, Iliodromiti Z, Lambrinoudaki I, Sifakis S, Creatsas G. Previous gestational diabetes mellitus and markers of cardiovascular risk. Int J Endocrinol. 2012;2012:458610.
9. Shah BR, Retnakaran R, Booth GL. Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. Diabetes Care. 2008;31(8):1668–9.
10. National Cholesterol Education Program (NCEP) Expert Panel on Detection. Evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III): Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation. 2002;106(25):3143–421.
11. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med. 1998;339(4):229–34.
12. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002;288(21):2709–16.
13. Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, et al. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the west of Scotland coronary prevention study. Circulation. 2003;108(4):414–19.
14. Vanhala MJ, Kumpusalo EA, Pitkäjärvi TK, Takala JK. Metabolic syndrome in a middle-aged finnish population. J Cardiovasc Risk. 1997;4(4):291–5.
15. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care. 2001;24(4):683–9.
16. Anastasiou E, Lekakis JP, Alevizaki M, Papamichael CM, Megas J, Souvatzoglou A, et al. Impaired endothelium-dependent vasodilatation in women with previous gestational diabetes. Diabetes Care. 1998;21(12):2111–15.
17. Hannemann MM, Liddell WG, Shore AC, Clark PM, Tooke JE. Vascular function in women with previous gestational diabetes mellitus. J Vasc Res. 2002;39(4):311–19.

18. Heitritter SM, Solomon CG, Mitchell GF, Skali-Ounis N, Seely EW. Subclinical inflammation and vascular dysfunction in women with previous gestational diabetes mellitus. J Clin Endocrinol Metab. 2005;90(7):3983–8.
19. Gobl CS, Bozkurt L, Yarragudi R, Prikoszovich T, Tura A, Pacini G, et al. Biomarkers of endothelial dysfunction in relation to impaired carbohydrate metabolism following pregnancy with gestational diabetes mellitus. Cardiovasc Diabetol. 2014;13(1):138.
20. Akinci B, Celtik A, Genc S, Yener S, Demir T, Secil M, et al. Evaluation of postpartum carbohydrate intolerance and cardiovascular risk factors in women with gestational diabetes. Gynecol Endocrinol. 2011;27(5):361–7.
21. Di Cianni G, Lencioni C, Volpe L, Ghio A, Cuccuru I, Pellegrini G, et al. C-reactive protein and metabolic syndrome in women with previous gestational diabetes. Diabetes Metab Res Rev. 2007;23(2):135–40.
22. Tam WH, Yang XL, Chan JC, Ko GT, Tong PC, Ma RC, et al. Progression to impaired glucose regulation, diabetes and metabolic syndrome in Chinese women with a past history of gestational diabetes. Diabetes Metab Res Rev. 2007;23(6):485–9.
23. Tam WH, Ma RC, Yang X, Ko GT, Lao TT, Chan MH, et al. Cardiometabolic risk in Chinese women with prior gestational diabetes: a 15-year follow-up study. Gynecol Obstet Invest. 2012;73(2):168–76.
24. Kaaja R, Alenius H, Kinnunen T, Komulainen J, Peränen N, Rönnemaa T, et al.: Gestational diabetes (online). Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim, the Medical Advisory Board of the Finnish Diabetes Association and the Finnish Gynecological Association, 2013 (Updated 25.6.2013). Available online at: www.kaypahoito.fi.
25. Cereda E, Malavazos AE, Caccialanza R, Rondanelli M, Fatati G, Barichella M. Weight cycling is associated with body weight excess and abdominal fat accumulation: a cross-sectional study. Clin Nutr. 2011;30(6):718–23.
26. Saquib N, Stefanick ML, Natarajan L, Pierce JP. Mortality risk in former smokers with breast cancer: pack-years vs. Smoking status. Int J Cancer. 2013;133(10):2493–7.
27. World Medical Association Inc. Declaration of Helsinki Ethical principles for medical research involving human subjects. J Indian Med Assoc. 2009;107(6):403–5.
28. Borodulin K, Levälahti E, Saarikoski L, Lund L, Juolevi A, Grönholm M, et al.: Kansallinen FINRISKI 2012 -terveystutkimus - Osa 2: Tutkimuksen taulukkoliite. Available from Internet: http://urn.fi/URN:ISBN:978-952-302-054-2. *Juvenes Print - Suomen Yliopistopaino Oy, Tampere* 2014, 22/2013.
29. Colman E. Food and drug administration's obesity drug guidance document: a short history. Circulation. 2012;125(17):2156–64.
30. Mao H, Li Q, Gao S. Meta-analysis of the relationship between common type 2 diabetes risk gene variants with gestational diabetes mellitus. PLoS One. 2012;7(9):e45882.
31. Huopio H, Cederberg H, Vangipurapu J, Hakkarainen H, Pääkkönen M, Kuulasmaa T, et al. Association of risk variants for type 2 diabetes and hyperglycemia with gestational diabetes. Eur J Endocrinol. 2013;169:291–7.
32. Povel CM, Boer JM, Reiling E, Feskens JM. Genetic variants and the metabolic syndrome: a systematic review. Obes Rev. 2011;12:952–67.
33. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-xi:1–253.
34. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997;20(7):1183–97.

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 intimae 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/m^2). 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/m^2 (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.

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

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.

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

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 $\geq$ 30 kg/m^2), i.e. 43 GDM and 32 control participants. The non-obese group (BMI < 30 kg/m^2; 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).

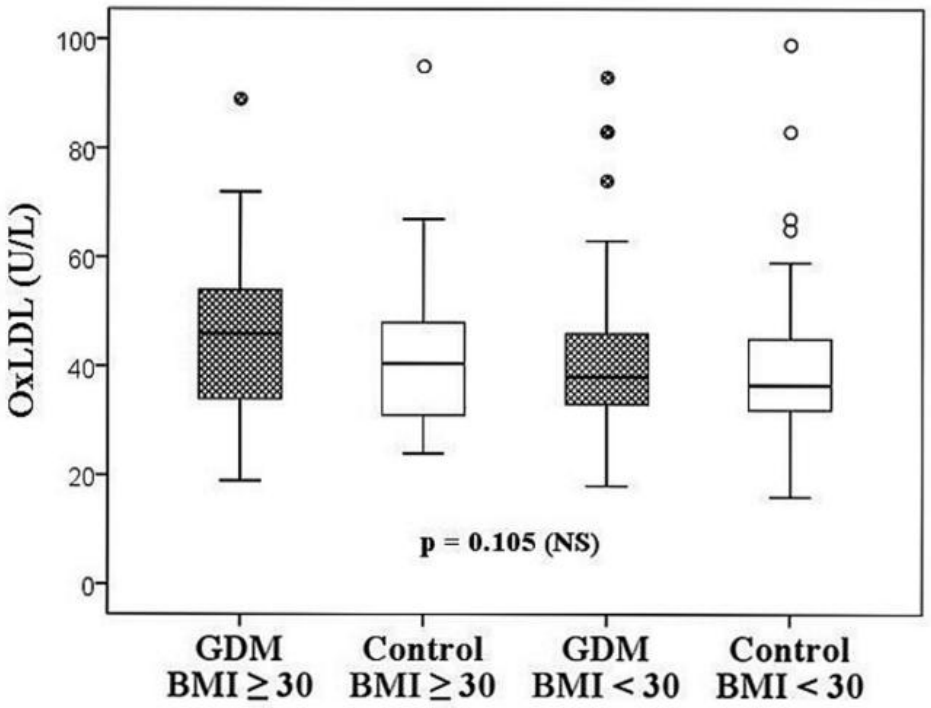

Figure 1 Plasma oxLDL concentrations in the four subgroups.

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).

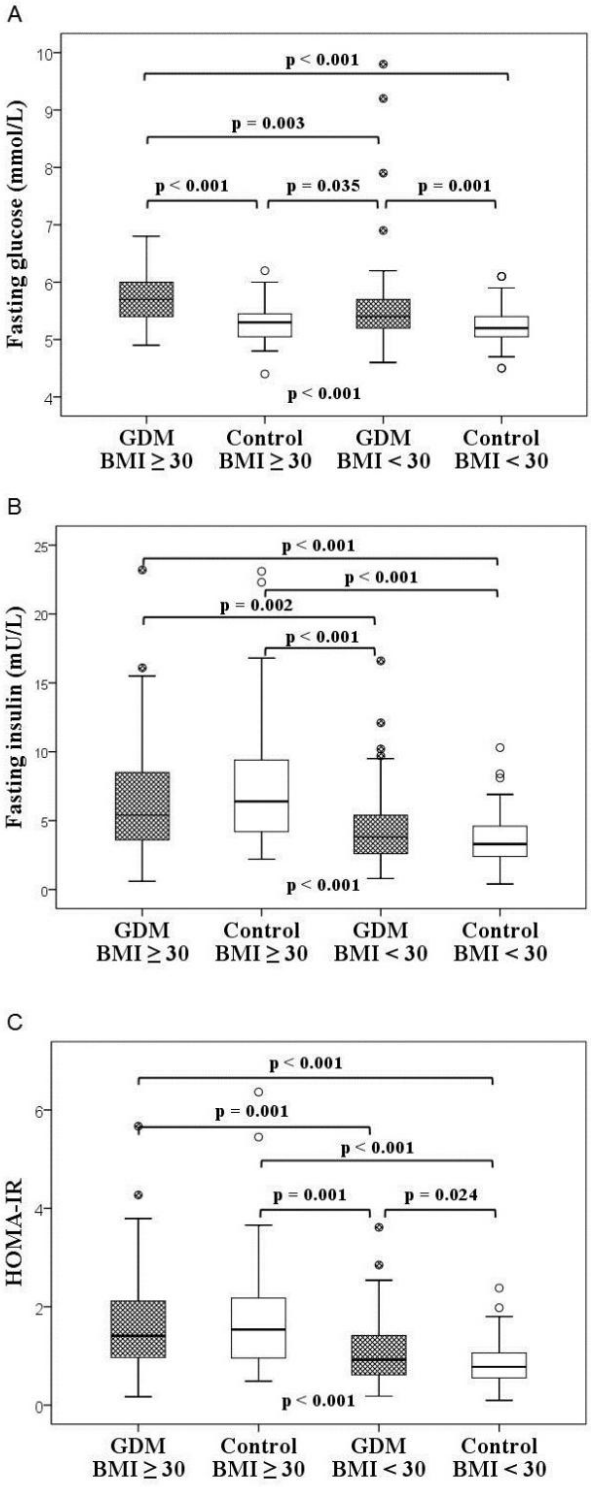

Figure 2 Fasting glucose (A), insulin (B) and HOMA-IR (C) in the four subgroups.

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.

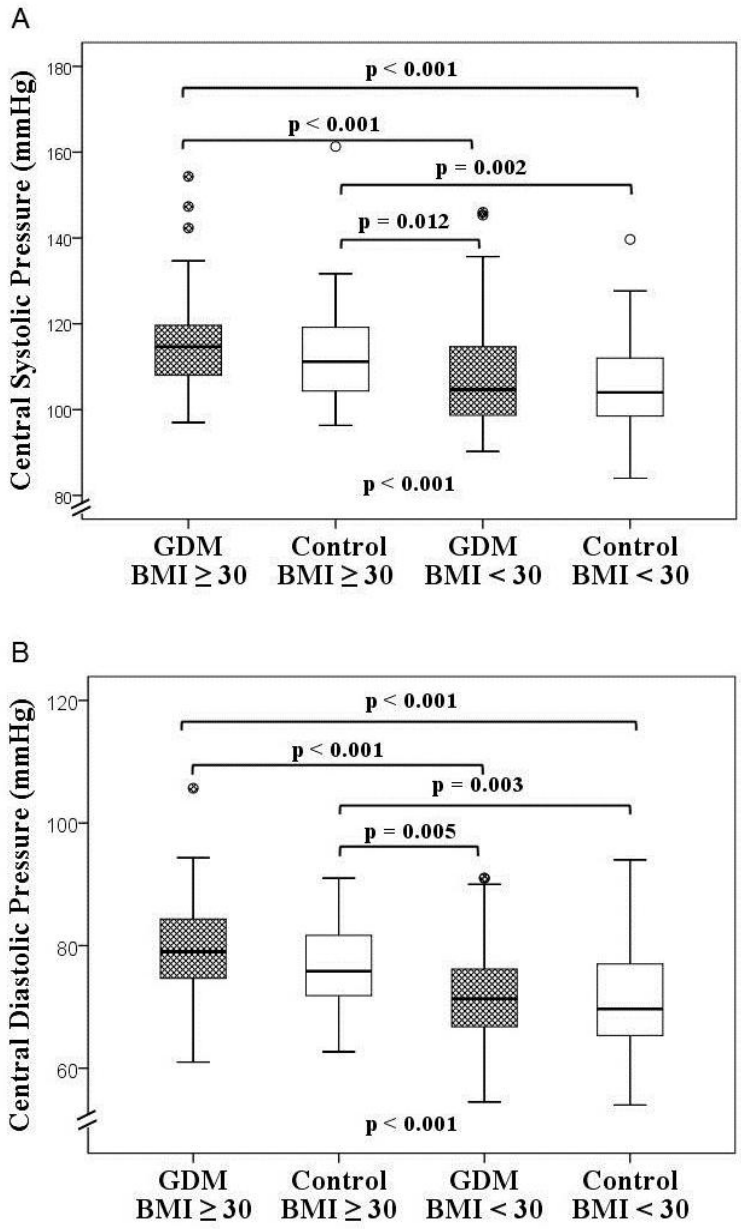

Figure 3 Central systolic (A) and diastolic pressures (B) in the four subgroups.

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 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 $p < 0.05$.

Parameters	**Covariates included in the model**	**R^2 for model**	**Global *p***	**Standardized β**	***p* value**
oxLDL		0.398	< 0.001		
	TC			0.659	< 0.001
	HDL-C			-0.319	< 0.001
HOMA-IR		0.346	< 0.001		
	BMI			0.394	< 0.001
	TC			0.220	< 0.001
	HDL-C			-0.223	< 0.001
	Heart rate			0.142	0.009
Systolic cBP		0.232	< 0.001		
	BMI			0.417	< 0.001
	Age			0.253	< 0.001
Diastolic cBP		0.227	< 0.001		
	BMI			0.375	< 0.001
	Age			0.184	0.002
	Heart rate			0.180	0.002

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 life-style intervention.

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References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014; 37 Suppl 1: S81-90.

2. Vuori E, Gissler M. Perinatal statistics: Parturients, deliveries and newborns 2014. ; Statistical report 19/2015. Helsinki: National Institute for Health and Welfare, 2015.

3. Kaaja R, Alenius H, Kinnunen T, Komulainen J, Peränen N, Rönnemaa T, *et al*. Gestational diabetes (online). current care guidelines. working group set up by the finnish medical society duodecim, the medical advisory board of the finnish diabetes association and the finnish gynecological association, 2013 [updated 25.6.2013]. available online at: Www.kaypahoito.fi.

4. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: A systematic review and meta-analysis. Lancet. 2009; 373: 1773-9.

5. Montecucco F, Steffens S, Mach F. Insulin resistance: A proinflammatory state mediated by lipid-induced signaling dysfunction and involved in atherosclerotic plaque instability. Mediators Inflamm. 2008; 2008: 767623.

6. Stocker R, Keaney JF,Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev. 2004; 84: 1381-478.

7. Sigurdardottir V, Fagerberg B, Hulthe J. Circulating oxidized low-density lipoprotein (LDL) is associated with risk factors of the metabolic syndrome and LDL size in clinically healthy 58-year-old men (AIR study). J Intern Med. 2002; 252: 440-7.

8. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, *et al*. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol. 2001; 21: 844-8.

9. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation. 1998; 98: 1487-94.

10. Njajou OT, Kanaya AM, Holvoet P, Connelly S, Strotmeyer ES, Harris TB, *et al*. Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the health, aging and body composition study. Diabetes Metab Res Rev. 2009; 25: 733-9.

11. Odegaar A, Jacobs DJ, Sanchez O, Goff DJ, Reiner A, Gross M. Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. Cardiovascular diabetology. 2016; 15: 51.

12. Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, *et al*. Central blood pressure measurements and antihypertensive therapy. A consensus document. Hypertension. 2007; 50: 154-60.

13. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, *et al*. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: Principal results of the conduit artery function evaluation (CAFE) study. Circulation. 2006; 113: 1213-25.

14. Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT, Tajik J. Noninvasive measurement of central vascular pressures with arterial tonometry: Clinical revival of the pulse pressure waveform? Mayo Clin Proc. 2010; 85: 460-72.

15. Dabelea D, Snell-Bergeon JK, Hartsfield CL, Bischoff KJ, Hamman RF, McDuffie RS, *et al*. Increasing prevalence of gestational diabetes mellitus (GDM) over time and by birth cohort: Kaiser permanente of colorado GDM screening program. Diabetes Care. 2005; 28: 579-84.

16. Vilmi-Kerälä T, Palomäki O, Vainio M, Uotila J, Palomäki A. The risk of metabolic syndrome after gestational diabetes mellitus – a hospital-based cohort study. Diabetology & Metabolic Syndrome. 2015; 7: 43.

17. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy: A world health organization guideline. Diabetes Res Clin Pract. 2014; 103: 341-63.

18. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, *et al*. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. Eur Heart J. 2006; 27: 2588-605.

19. World Medical Association Inc. Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Indian Med Assoc. 2009; 107: 403-5.

20. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009; 32: 1327–34.

21. Monzillo LU, Hamdy O. Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev. 2003; 61: 397-412.

22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28: 412-9.

23. Obesity: Preventing and managing the global epidemic. report of a WHO consultation. World Health Organ Tech Rep Ser. 2000; 894: i,xii, 1-253.

24. Cohn JN. Vascular wall function as a risk marker for cardiovascular disease. J Hypertens Suppl. 1999; 17: S41-4.

25. Pohjantähti-Maaroos H, Palomäki A, Kankkunen P, Laitinen R, Husgafvel S, Oksanen K. Circulating oxidized low-density lipoproteins and arterial elasticity: Comparison between men with metabolic syndrome and physically active counterparts. Cardiovasc Diabetol. 2010; 9: 41,2840-9-41.

26. Benhalima K, Leuridan L, Calewaert P, Devlieger R, Verhaeghe J, Mathieu C. Glucose intolerance after a recent history of gestational diabetes. Int J Endocrinol. 2014; 2014: 727652.

27. Antuna-Puente B, Disse E, Rabasa-Lhoret R, Laville M, Capeau J, Bastard JP. How can we measure insulin sensitivity/resistance? Diabetes Metab. 2011; 37: 179-88.

28. Lann D, LeRoith D. Insulin resistance as the underlying cause for the metabolic syndrome. Med Clin North Am. 2007; 91: 1063,77, viii.

29. Saucedo R, Zarate A, Basurto L, Hernandez M, Puello E, Galvan R, *et al*. Relationship between circulating adipokines and insulin resistance during pregnancy and postpartum in women with gestational diabetes. Arch Med Res. 2011; 42: 318-23.

30. Nurnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schafers RF. Augmentation index is associated with cardiovascular risk. J Hypertens. 2002; 20: 2407-14.

31. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, *et al*. Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation. 2004; 109: 184-9.

32. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: A systematic review and meta-analysis. Eur Heart J. 2010; 31: 1865-71.

33. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: Current evidence and clinical importance. Eur Heart J. 2014; 35: 1719-25.

34. Lekva T, Bollerslev J, Norwitz ER, Aukrust P, Henriksen T, Ueland T. Aortic stiffness and cardiovascular risk in women with previous gestational diabetes mellitus. PLoS One. 2015; 10: e0136892.

35. Mai C, Hou M, Chen R, Duan D, Xu H, Lin X, *et al*. Cardiovascular risk factors in chinese women with a history of gestational diabetes mellitus. International Journal of Clinical and Experimental Medicine. 2015; 8 (11): 21694-8.

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ORIGINAL INVESTIGATION

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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 $\geq$5.3 mmol/L when fasting, $\geq$10.0 mmol/L at 1 h or $\geq$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 $\geq$5.1 mmol/L when fasting, $\geq$10.0 mmol/L at 1 h or $\geq$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 $\geq$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/m^2).

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/m^2 [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 tested 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 1 Basic clinical characteristics of women with GDM and controls

	GDM	Controls	*p* value
Average time since delivery, years	3.7 ± 1.0	3.7 ± 0.9	0.818
Age, years	35.8 ± 4.4	35.9 ± 4.6	0.854
Primiparous, n (%)	23 (19.2%)	23 (19.2%)	1.000
Therapy of GDM during pregnancy			
Insulin, n (%)	24 (20.0%)		
Metformin, n (%)	1 (0.8%)		
Dietary therapy, n (%)	95 (79.2%)		
Pack-years of smoking	3.8 ± 6.0	2.4 ± 4.6	0.012
During the previous month, history of			
Respiratory infection, n (%)	45 (37.5%)	44 (36.7%)	0.854
Other infectious disease, n (%)	18 (15.0%)	10 (8.3%)	0.053
Trauma, n (%)	9 (7.5%)	5 (4.2%)	0.264
BMI, kg/m^2	28.3 ± 5.0	27.5 ± 5.4	0.069
Systolic BP, mmHg	122.4 ± 12.5	119.0 ± 11.5	0.034
Diastolic BP, mmHg	73.5 ± 9.0	71.8 ± 8.7	0.176
Heart rate, beats per minute	65.9 ± 9.1	63.8 ± 9.6	0.017
TC, mmol/L	4.7 ± 0.9	4.6 ± 0.8	0.329
F-Gluc, mmol/L	5.6 ± 0.6	5.3 ± 0.3	<0.001
F-Insu, mU/L	5.2 ± 3.6	4.6 ± 3.6	0.087

Data are presented as mean ± SD if not mentioned otherwise

BMI body mass index, *BP* blood pressure, *F-Gluc* fasting glucose, *F-Insu* fasting insulin, *TC* total cholesterol

Table 2 Results of primary analyses of GDM and control groups

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

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 3 Results of stepwise multiple linear regression analyses

Parameters	Covariates included in the model	R^2 for model	Global *p*	Standardized β	*p* value
hsCRP		0.096	<0.001		
	BMI			0.259	<0.001
MMP-8		0.138	<0.001		
	hsCRP			0.312	<0.001
	Previous GDM			−0.137	0.025
	TC			0.129	0.036
TIMP-1		0.067	0.003		
	Previous GDM			0.157	0.015
	BMI			0.149	0.025
	Heart rate			−0.132	0.044
C1		0.524	<0.001		
	Systolic BP			−0.602	<0.001
	Heart rate			−0.347	<0.001
	BMI			0.232	<0.001
	Time after the index pregnancy			−0.095	0.041
C2		0.317	<0.001		
	Systolic BP			−0.345	<0.001
	Heart rate			−0.312	<0.001
	BMI			0.286	<0.001
	Age			−0.191	0.001
	Pack-years of smoking			−0.144	0.012
PWV		0.470	<0.001		
	Systolic BP			0.534	<0.001
	Age			0.230	<0.001
	F-Insu			0.191	<0.001
	Previous GDM			0.105	0.026
	Time after the index pregnancy			−0.102	0.040

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 $p < 0.05$

BMI body mass index, *BP* blood pressure, *F-Insu* fasting insulin, *GDM* gestational diabetes mellitus, *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

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 $\geq$ 30 kg/m^2); 43 GDM and 32 control participants. The non-obese group (BMI < 30 kg/m^2; $n = 165$) 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). Between the subgroups, there were no differences in the MMP-8/TIMP-1 or MMP-9/TIMP-1 ratio either (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).

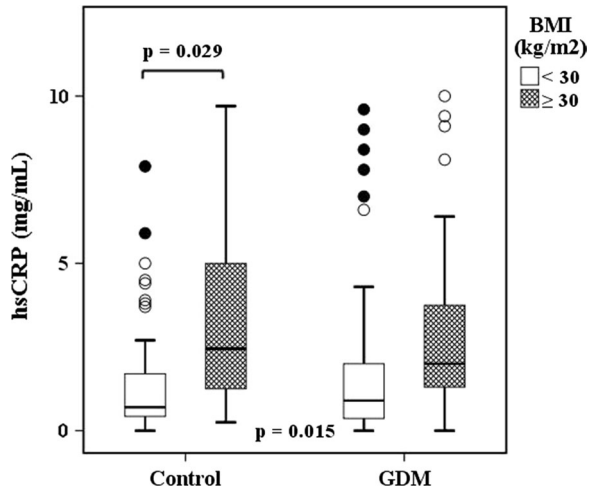

Fig. 1 Serum concentrations of hsCRP in the four subgroups. Median values (minimum, maximum) of hsCRP: among obese GDM women 2.1 (0.0, 12.4) mg/mL, obese control women 2.1 (0.3, 18.5) mg/mL, non-obese GDM women 0.9 (0.0, 32.3) mg/mL, and non-obese control women 0.7 (0.0, 25.7) mg/mL. Values of more than 10 mg/mL were measured by turbidimetric immunoassay. The overall *p* value is given at the *bottom*. Individual *p* values for pairwise comparisons are also presented

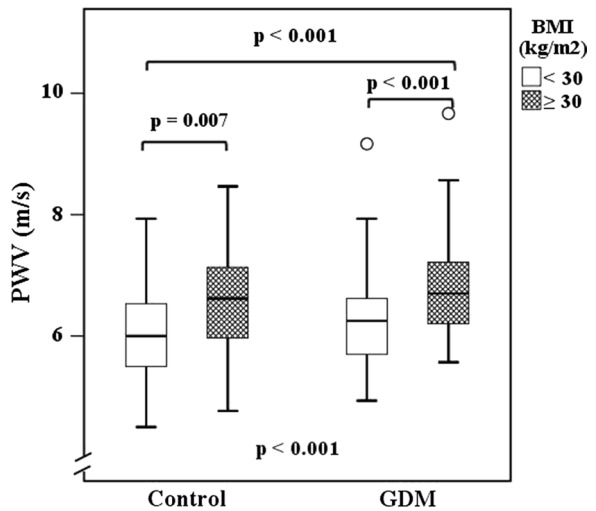

Fig. 2 PWV in the four subgroups. Median values (minimum, maximum) of PWV: among obese GDM women 6.8 (5.6, 9.7) m/s, obese control women 6.6 (4.8, 8.5) m/s, non-obese GDM women 6.3 (4.9, 9.2) m/s, and non-obese control women 6.0 (4.5, 7.9) m/s. The overall *p* value is given at the *bottom*. Individual *p* values for pairwise comparisons are also presented

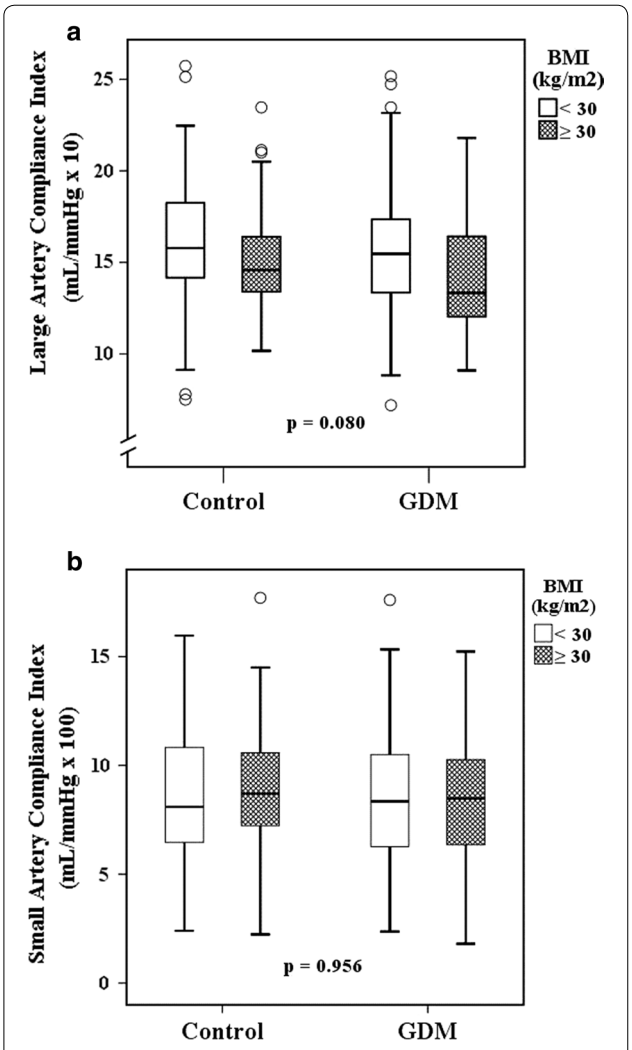

Fig. 3 Large (**a**) and small (**b**) artery compliance index values in the four subgroups. **a** Median values (minimum, maximum) of the large-artery compliance index (C1): among obese GDM women 13.3 (9.1, 21.8) mL/mmHg × 10, obese control women 14.7 (10.2, 23.5) mL/mmHg × 10, non-obese GDM women 15.2 (7.2, 25.2) mL/mmHg × 10, and non-obese control women 15.9 (7.5, 25.7) mL/mmHg × 10. The overall *p* value is given. **b** Median values (minimum, maximum) of the small-artery compliance index (C2): among obese GDM women 8.8 (2.8, 15.2) mL/mmHg × 100, obese control women 8.6 (2.2, 17.7) mL/mmHg × 100, non-obese GDM women 8.1 (1.8, 17.6) mL/mmHg × 100, and non-obese control women 8.1 (2.4, 16.0) mL/mmHg × 100. The overall *p* value is given

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 $\geq$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 $\geq$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, JU 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

1. Dabelea D, Snell-Bergeon JK, Hartsfield CL, Bischoff KJ, Hamman RF, McDuffie RS. Kaiser Permanente of Colorado GDM Screening Program: increasing prevalence of gestational diabetes mellitus (GDM) over time and by birth cohort: Kaiser Permanente of Colorado GDM Screening Program. Diabetes Care. 2005;28(3):579–84.
2. Vuori E, Gissler M. Perinatal statistics: parturients, deliveries and newborns 2015. Statistical report 16/2016. Helsinki: National Institute for Health and Welfare; 2016.
3. Gobl CS, Bozkurt L, Yarragudi R, Prikoszovich T, Tura A, Pacini G, Koppensteiner R, Kautzky-Willer A. Biomarkers of endothelial dysfunction in relation to impaired carbohydrate metabolism following pregnancy with gestational diabetes mellitus. Cardiovasc Diabetol. 2014;13(1):138.
4. Heitritter SM, Solomon CG, Mitchell GF, Skali-Ounis N, Seely EW. Subclinical inflammation and vascular dysfunction in women with previous gestational diabetes mellitus. J Clin Endocrinol Metab. 2005;90(7):3983–8.
5. Vrachnis N, Augoulea A, Iliodromiti Z, Lambrinoudaki I, Sifakis S, Creatsas G. Previous gestational diabetes mellitus and markers of cardiovascular risk. Int J Endocrinol. 2012;2012:458610.

6. Shah BR, Retnakaran R, Booth GL. Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. Diabetes Care. 2008;31(8):1668–9.
7. Goueslard K, Cottenet J, Mariet AS, Giroud M, Cottin Y, Petit JM, Quantin C. Early cardiovascular events in women with a history of gestational diabetes mellitus. Cardiovasc Diabetol. 2016;15:15.
8. Retnakaran R, Shah BR. Role of type 2 diabetes in determining retinal, renal, and cardiovascular outcomes in women with previous gestational diabetes mellitus. Diabetes Care. 2017;40(1):101–8.
9. Karadeniz M, Duran M, Akyel A, Yarlioglues M, Ocek AH, Celik IE, Kilic A, Yalcin AA, Ergun G, Murat SN. High sensitive crp level is associated with intermediate and high syntax score in patients with acute coronary syndrome. Int Heart J. 2015;56(4):377–80.
10. Lenglet S, Mach F, Montecucco F. Role of matrix metalloproteinase-8 in atherosclerosis. Mediat Inflamm. 2013;2013:659282.
11. Sorsa T, Tjaderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL, Mäntylä P. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Ann Med. 2006;38(5):306–21.
12. Lauhio A, Salo T, Ding Y, Konttinen YT, Nordström D, Tschesche H, Lähdevirta J, Golub LM, Sorsa T. In vivo inhibition of human neutrophil collagenase (MMP-8) activity during long-term combination therapy of doxycycline and non-steroidal anti-inflammatory drugs (NSAID) in acute reactive arthritis. Clin Exp Immunol. 1994;98(1):21–8.
13. Lauhio A, Konttinen YT, Tschesche H, Nordström D, Salo T, Lähdevirta J, Golub LM, Sorsa T. Reduction of matrix metalloproteinase 8-neutrophil collagenase levels during long-term doxycycline treatment of reactive arthritis. Antimicrob Agents Chemother. 1994;38(2):400–2.
14. Lauhio A, Salo T, Tjaderhane L, Lähdevirta J, Golub LM, Sorsa T. Tetracyclines in treatment of rheumatoid arthritis. Lancet. 1995;346(8975):645–6.
15. Lauhio A, Saikku P, Salo T, Tschesche H, Lähdevirta J, Sorsa T. Combination treatment in Chlamydia-triggered reactive arthritis: comment on the article by Carter et al. Arthritis Rheum. 2011;63(1):305–7 **(author reply 307–8)**.
16. Lauhio A, Hästbacka J, Pettilä V, Tervahartiala T, Karlsson S, Varpula T, Varpula M, Ruokonen E, Sorsa T, Kolho E. Serum MMP-8, -9 and TIMP-1 in sepsis: high serum levels of MMP-8 and TIMP-1 are associated with fatal outcome in a multicentre, prospective cohort study. Hypothetical impact of tetracyclines. Pharmacol Res. 2011;64(6):590–4.
17. Lauhio A, Färkkilä E, Pietiläinen KH, Åström P, Winkelmann A, Tervahartiala T, Pirilä E, Rissanen A, Kaprio J, Sorsa TA, Salo T. Association of MMP-8 with obesity, smoking and insulin resistance. Eur J Clin Investig. 2016;46(9):757–65.
18. Rautelin HI, Oksanen AM, Veijola LI, Sipponen PI, Tervahartiala TI, Sorsa TA, Lauhio A. Enhanced systemic matrix metalloproteinase response in *Helicobacter pylori* gastritis. Ann Med. 2009;41(3):208–15.
19. Cena JJ, Lalu MM, Cho WJ, Chow AK, Bagdan ML, Daniel EE, Castro MM, Schulz R. Inhibition of matrix metalloproteinase activity in vivo protects against vascular hyporeactivity in endotoxemia. Am J Physiol Heart Circ Physiol. 2010;298(1):H45–51.
20. Siasos G, Tousoulis D, Kioufis S, Oikonomou E, Siasou Z, Limperi M, Papavassiliou AG, Stefanadis C. Inflammatory mechanisms in atherosclerosis: the impact of matrix metalloproteinases. Curr Top Med Chem. 2012;12(10):1132–48.
21. Paim LR, Schreiber R, Matos-Souza JR, Silva AA, Campos LF, Azevedo ER, Alonso K, de Rossi G, Etchebehere M, Gorla JI, Cliquet A Jr, Nadruz W Jr. Oxidized low-density lipoprotein, matrix-metalloproteinase-8 and carotid atherosclerosis in spinal cord injured subjects. Atherosclerosis. 2013;231(2):341–5.
22. Goncalves FM, Jacob-Ferreira AL, Gomes VA, Casella-Filho A, Chagas AC, Marcaccini AM, Gerlach RF, Tanus-Santos JE. Increased circulating levels of matrix metalloproteinase (MMP)-8, MMP-9, and pro-inflammatory markers in patients with metabolic syndrome. Clin Chim Acta. 2009;403(1–2):173–7.
23. Pussinen PJ, Sarna S, Puolakkainen M, Ohlin H, Sorsa T, Pesonen E. The balance of serum matrix metalloproteinase-8 and its tissue inhibitor in acute coronary syndrome and its recurrence. Int J Cardiol. 2013;167(2):362–8.
24. Sorsa T, Tervahartiala T, Leppilahti J, Hernandez M, Gamonal J, Tuomainen AM, Lauhio A, Pussinen PJ, Mäntylä P. Collagenase-2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. Pharmacol Res. 2011;63(2):108–13.
25. Tuomainen AM, Nyyssönen K, Laukkanen JA, Tervahartiala T, Tuomainen TP, Salonen JT, Sorsa T, Pussinen PJ. Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men. Arterioscler Thromb Vasc Biol. 2007;27(12):2722–8.
26. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA. 2004;291(16):1978–86.
27. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. 2002;39(1):10–5.
28. Nigam A, Mitchell GF, Lambert J, Tardif J. Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. Am J Cardiol. 2003;92(4):395–9.
29. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H. European network for non-invasive investigation of large arteries: expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588–605.
30. Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, Wang J, Wilkinson IB, Williams B, Vlachopoulos C. Central blood pressure measurements and antihypertensive therapy. A consensus document. Hypertension. 2007;50:154–60.
31. Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT, Tajik J. Noninvasive measurement of central vascular pressures with arterial tonometry: clinical revival of the pulse pressure waveform? Mayo Clin Proc. 2010;85(5):460–72.
32. Kim YK. Impact of the metabolic syndrome and its components on pulse wave velocity. Korean J Intern Med. 2006;21(2):109–15.
33. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation. 2002;106(16):2085–90.
34. Kormi I, Alfakry H, Tervahartiala T, Pussinen PJ, Sinisalo J, Sorsa T. The effect of prolonged systemic doxycycline therapy on serum tissue degrading proteinases in coronary bypass patients: a randomized, double-masked, placebo-controlled clinical trial. Inflamm Res. 2014;63(5):329–34.
35. Payne JB, Golub LM, Stoner JA, Lee HM, Reinhardt RA, Sorsa T, Slepian MJ. The effect of subantimicrobial-dose-doxycycline periodontal therapy on serum biomarkers of systemic inflammation: a randomized, double-masked, placebo-controlled clinical trial. J Am Dent Assoc. 2011;142(3):262–73.
36. Hayakawa T, Yamashita K, Tanzawa K, Uchijima E, Iwata K. Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. A possible new growth factor in serum. FEBS Lett. 1992;298(1):29–32.
37. Moore CS, Crocker SJ. An alternate perspective on the roles of TIMPs and MMPs in pathology. Am J Pathol. 2012;180(1):12–6.
38. Stetler-Stevenson WG. Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. Sci Signal. 2008;1(27):re6.
39. Kaaja R, Alenius H, Kinnunen T, Komulainen J, Peränen N, Rönnemaa T, Saramies J, Soukka H, Teramo K, Vuorela P, Vääräsmäki M. Gestational diabetes (online). Current care guidelines. Working group set up by the Finnish Medical Society Duodecim, the Medical Advisory Board of the Finnish Diabetes Association and the Finnish Gynecological Association; 2013. http://www.kaypahoito.fi. Accessed 25 June 2013.
40. Vilmi-Kerälä T, Palomäki O, Vainio M, Uotila J, Palomäki A. The risk of metabolic syndrome after gestational diabetes mellitus—a hospital-based cohort study. Diabetol Metab Syndr. 2015;7:43.
41. Report of a World Health Organization Consultation. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy: a World Health Organization guideline. Diabetes Res Clin Pract. 2014;103(3):341–63.
42. Saquib N, Stefanick ML, Natarajan L, Pierce JP. Mortality risk in former smokers with breast cancer: pack-years vs. smoking status. Int J Cancer. 2013;133(10):2493–7.
43. World Medical Association Inc. Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Indian Med Assoc. 2009;107(6):403–5.
44. Chenillot O, Henny J, Steinmetz J, Herbeth B, Wagner C, Siest G. High sensitivity C-reactive protein: biological variations and reference limits. Clin Chem Lab Med. 2000;38(10):1003–11.

45. Sanchez A, Mirabel JL, Barrenechea E, Eugui J, Puelles A, Castaneda A. Evaluation of an improved immunoturbidimetic assay for serum C-reactive protein on a COBAS INTEGRA 400 Analyzer. Clin Lab. 2002;48(5–6):313–7.
46. Cohn JN, Finkelstein S, McVeigh G, Morgan D, LeMay L, Robinson J, Mock J. Noninvasive pulse wave analysis for the early detection of vascular disease. Hypertension. 1995;26(3):503–8.
47. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser, vol 894. 2000; p. i–xii, 1–253.
48. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002;347(20):1557–65.
49. Berg G, Miksztowicz V, Schreier L. Metalloproteinases in metabolic syndrome. Clin Chim Acta. 2011;412(19–20):1731–9.
50. Ozuguz U, Isik S, Berker D, Arduc A, Tutuncu Y, Akbaba G, Gokay F, Guler S. Gestational diabetes and subclinical inflammation: evaluation of first year postpartum outcomes. Diabetes Res Clin Pract. 2011;94(3):426–33.
51. Lekva T, Michelsen AE, Bollerslev J, Norwitz ER, Aukrust P, Henriksen T, Ueland T. Low circulating pentraxin 3 levels in pregnancy is associated with gestational diabetes and increased apoB/apoA ratio: a 5-year follow-up study. Cardiovasc Diabetol. 2016;15:23.
52. Ajala O, Jensen LA, Ryan E, Chik C. Women with a history of gestational diabetes on long-term follow up have normal vascular function despite more dysglycemia, dyslipidemia and adiposity. Diabetes Res Clin Pract. 2015;110(3):309–14.
53. Lekva T, Bollerslev J, Norwitz ER, Aukrust P, Henriksen T. Aortic stiffness and cardiovascular risk in women with previous gestational diabetes mellitus. PLoS ONE. 2015;10(8):e0136892.
54. Tam WH, Ma RC, Chan JC, Lao TT, Chan MH, Li CY. PP103. Arterial stiffness in women with previous GDM—a follow up of Chinese HAPO study cohort. Pregnancy Hypertens. 2012;2(3):295.
55. Vilmi-Kerälä T, Palomäki O, Kankkunen P, Juurinen L, Uotila J, Palomäki A. Oxidized LDL, insulin resistance and central blood pressure after gestational diabetes mellitus. Acta Obstet Gynecol Scand. 2016;95(12):1425–32.
56. Acree LS, Montgomery PS, Gardner AW. The influence of obesity on arterial compliance in adult men and women. Vasc Med. 2007;12(3):183–8.
57. Huffman MD, Capewell S, Ning H, Shay CM, Ford ES, Lloyd-Jones DM. Cardiovascular health behavior and health factor changes (1988–2008) and projections to 2020: results from the National Health and Nutrition Examination Surveys. Circulation. 2012;125(21):2595–602.
58. Kim S, Kung C, Park JS, Lee SP, Kim HK, Ahn CW, Kim KR, Kang S. Normal-weight obesity is associated with increased risk of subclinical atherosclerosis. Cardiovasc Diabetol. 2015;14:58.
59. Bouchi R, Ohara N, Asakawa M, Nakano Y, Takeuchi T, Murakami M, Sasahara Y, Numasawa M, Minami I, Izumiyama H, Hashimoto K, Yoshimoto T, Ogawa Y. Is visceral adiposity a modifier for the impact of blood pressure on arterial stiffness and albuminuria in patients with type 2 diabetes? Cardiovasc Diabetol. 2016;15:10.

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^{TM}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.

Matching parameter	**MetS (n = 27)**		**Control (n = 27)**				**P value**
	Mean	**SD**	**Mean**	**SD**	**Difference**	**95% CI**	
Age, years	36.8	4.7	36.6	4.5	0.2	-2.3, 2.7	0.880
Previous GDM, n (%)	19	70	19	70			1.000
TC, mmol/L	5.1	1.2	5.2	0.9	-0.1	-0.7, 0.5	0.851
LDL-C, mmol/L	3.4	0.9	3.3	0.8	0.1	-0.4, 0.5	0.768

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^{TM}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/m^2).

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^{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® SPSS® 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.

Determinant of MetS	**MetS (n = 27)**		**Control (n = 27)**				**P value**
	Mean	**SD**	**Mean**	**SD**	**Difference**	**95% CI**	
Waist circumference, cm	107.7	11.0	97.8	14.1	9.9	2.6, 17.2	0.010
Systolic BP, mmHg	135.7	13.6	125.9	18.7	9.8	0.2, 19.4	0.021
Diastolic BP, mmHg	78.4	8.1	73.0	12.1	5.4	-0.6 11.4	0.053
Fasting glucose, mmol/L	5.7	0.6	5.4	0.4	0.3	0.0, 0.6	0.029
T2DM, n (%)	1*	4	0	0			1.000
TG, mmol/L	1.7	0.9	1.0	0.3	0.7	0.4, 1.1	< 0.001
HDL-C, mmol/L	1.2	0.2	1.6	0.2	-0.3	-0.5, -0.2	< 0.001

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.

	MetS n = 27		Control n = 27				P value
	Mean	**SD**	**Mean**	**SD**	**Difference**	**95% CI**	
Pack-years of smoking	4.1	8.7	1.9	4.8	2.1	-1.8, 6.0	0.276
Alcohol intake, g/day	1.1	1.4	1.5	1.6	-0.6	-1.4, 0.1	0.242
Lifetime weight loss, kg	30.4	31.4	28.0	35.2	2.4	-18.7, 23.6	0.657
BMI, kg/m^2	33.5	6.2	28.9	5.0	4.6	1.2, 7.9	0.010
Clinical chemistry							
Hemoglobin, g/L	138.2	6.9	130.5	9.1	7.2	2.5, 11.9	0.004
hsCRP, mg/L	3.6	4.1	3.7	5.2	-0.1	-2.7, 2.6	0.516
oxLDL, U/L	48.3	14.6	48.0	17.1	0.3	-8.1, 8.7	0.942
F-insu, mU/L	9.0	5.9	6.4	4.3	2.6	-0.5, 5.7	0.073
ALAT, U/L	32.3	24.1	22.2	20.5	10.3	0.6, 19.5	0.022
Crea, µmol/L	65.3	9.0	64.6	5.4	0.7	-3.8, 5.2	0.748
HOMA-IR	2.3	1.5	1.6	1.1	0.7	-0.1, 1.5	0.046

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).

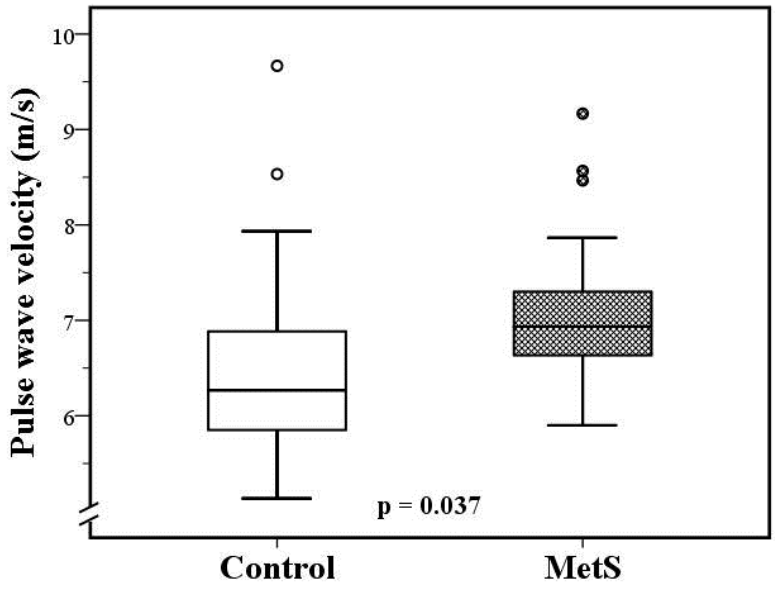

Figure 1 PWV in the MetS women and their matched controls without the syndrome.

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).

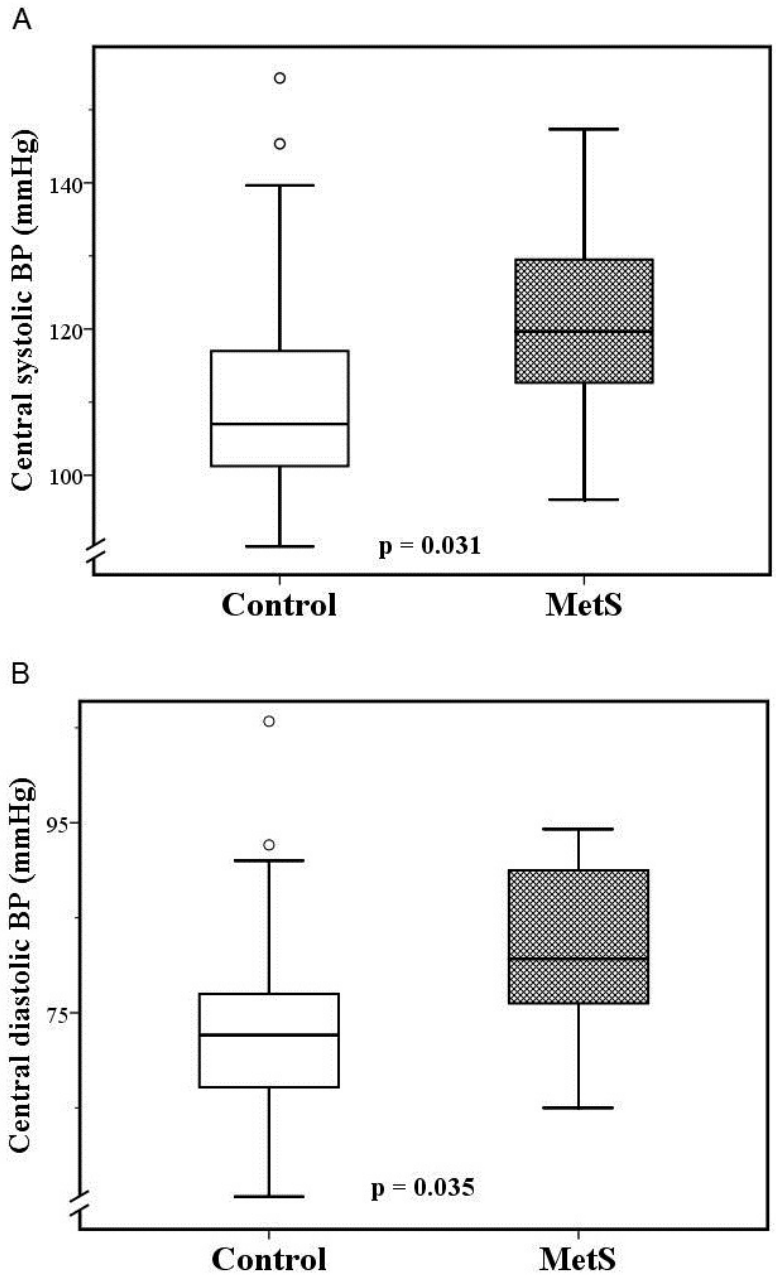

Figure 2 Central systolic (A) and diastolic pressures (B) in the MetS women and their matched controls without the syndrome.

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).

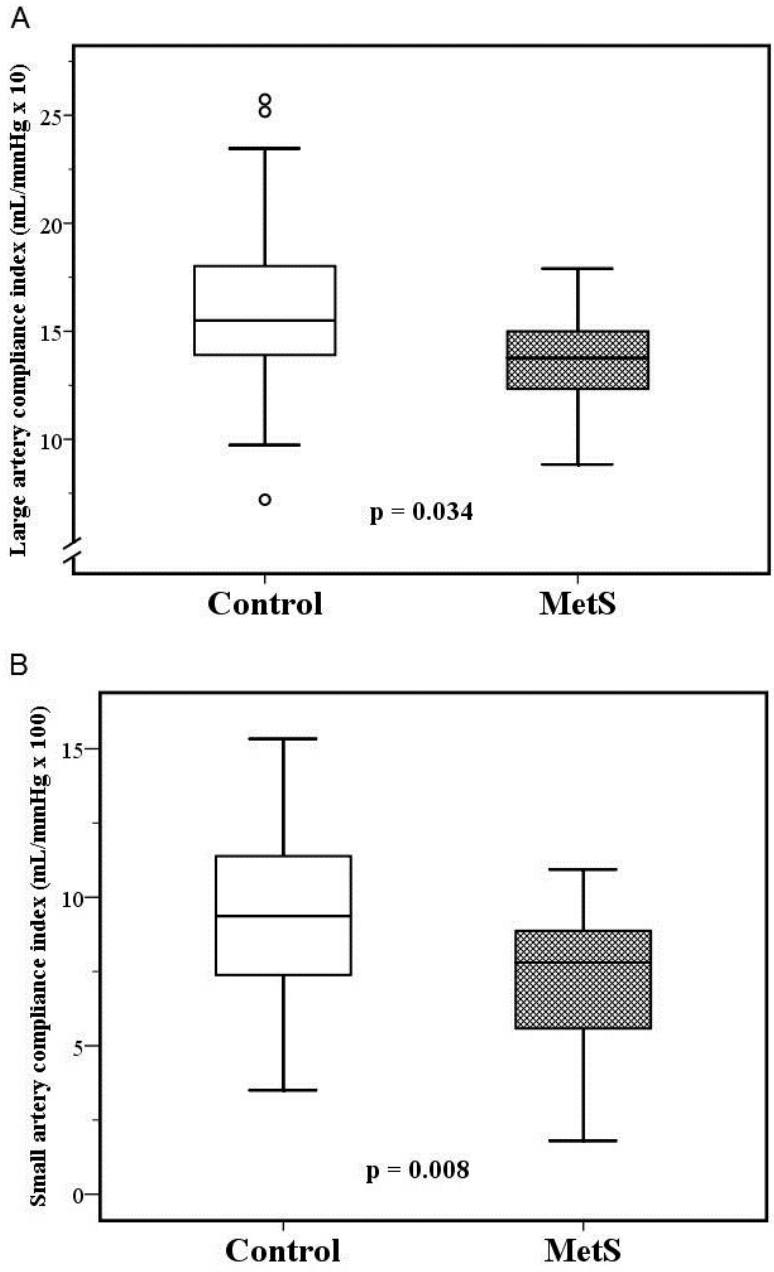

Figure 3 Large- (A) and small-artery (B) compliance index values in the MetS women and their matched controls without the syndrome.

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-Maaroos 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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References

(1) Reaven G. Role of insulin resistance in human disease. Diabetes 1988;37:595-607.

(2) National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;10625:3143-3421.

(3) Xu Y, Shen S, Sun L, Yang H, Jin B, Cao X. Metabolic syndrome risk after gestational diabetes: a systematic review and meta-analysis. PLoS One 2014;91:e87863.

(4) Trevisan M, Liu J, Bahsas FB, Menotti A. Syndrome X and Mortality: A Population-based Study. American Journal of Epidemiology 1998;148:958-66.

(5) Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;244:683-89.

(6) Mente A, Yusuf S, Islam S, McQueen MJ, Tanomsup S, Onen CL, et al. Metabolic syndrome and risk of acute myocardial infarction a case-control study of 26,903 subjects from 52 countries. J Am Coll Cardiol 2010;5521:2390-98.

(7) Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;3809859:2095-2128.

(8) Rich-Edwards JW, Fraser A, Lawlor DA, Catov JM. Pregnancy characteristics and women's future cardiovascular health: an underused opportunity to improve women's health? Epidemiol Rev 2014;36:57-70.

(9) Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. Circulation 2011;12311:1243-62.

(10) Shaw LJ, Bairey Merz CN, Pepine CJ, Reis SE, Bittner V, Kelsey SF, et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part I: gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. J Am Coll Cardiol 2006;473 Suppl:S4-S20.

(11) Akinci B, Celtik A, Genc S, Yener S, Demir T, Secil M, et al. Evaluation of postpartum carbohydrate intolerance and cardiovascular risk factors in women with gestational diabetes. Gynecol Endocrinol 2011;275:361-67.

(12) Lauenborg J, Mathiesen E, Hansen T, Glumer C, Jorgensen T, Borch-Johnsen K, et al. The prevalence of the metabolic syndrome in a danish population of women with previous gestational diabetes mellitus is three-fold higher than in the general population. J Clin Endocrinol Metab 2005;907:4004-10.

(13) Di Cianni G, Lencioni C, Volpe L, Ghio A, Cuccuru I, Pellegrini G, et al. C-reactive protein and metabolic syndrome in women with previous gestational diabetes. Diabetes Metab Res Rev 2007;232:135-40.

(14) Vilmi-Kerälä T, Palomäki O, Vainio M, Uotila J, Palomäki A. The risk of metabolic syndrome after gestational diabetes mellitus – a hospital-based cohort study. Diabetology & Metabolic Syndrome 2015;7:43.

(15) Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;391:10-15.

(16) Nigam A, Mitchell GF, Lambert J, Tardif J. Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. The American Journal of Cardiology 2003;924:395-99.

(17) Hodes RJ, Lakatta EG, McNeil CT. Another modifiable risk factor for cardiovascular disease? Some evidence points to arterial stiffness. J Am Geriatr Soc 1995;435:581-82.

(18) Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;2721:2588-2605.

(19) Kelly R, Hayward C, Avolio A, O'Rourke M. Noninvasive determination of age-related changes in the human arterial pulse. Circulation 1989;806:1652-59.

(20) Nichols WW. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. Am J Hypertens 2005;181 Pt 2:3S-10S.

(21) Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, et al. Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation 1993;884 Pt 1:1456-62.

(22) McVeigh GE, Hamilton PK, Morgan DR. Evaluation of mechanical arterial properties: clinical, experimental and therapeutic aspects. Clin Sci (Lond) 2002;1021:51-67.

(23) Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT, Tajik J. Noninvasive Measurement of Central Vascular Pressures With Arterial Tonometry: Clinical Revival of the Pulse Pressure Waveform? Mayo Clin Proc 2010;855:460-72.

(24) Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation 2006;1139:1213-25.

(25) Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, et al. Central Blood Pressure Measurements and Antihypertensive Therapy. A Consensus Document. Hypertension 2007;50:154-60.

(26) Balkau B, Charles MA, Drivsholm T, Borch-Johnsen K, Wareham N, Yudkin JS, et al. Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. Diabetes Metab 2002;285:364-76.

(27) Bauduceau B, Vachey E, Mayaudon H, Burnat P, Dupuy O, Garcia C, et al. Should we have more definitions of metabolic syndrome or simply take waist measurement? Diabetes Metab 2007;335:333-39.

(28) Kahn R, Buse J, Ferrannini E, Stern M, American Diabetes Association, European Association for the Study of Diabetes. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2005;289:2289-2304.

(29) Woodward M, Tunstall-Pedoe H. The metabolic syndrome is not a sensible tool for predicting the risk of coronary heart disease. Eur J Cardiovasc Prev Rehabil 2009;162:210-14.

(30) Kaaja R, Alenius H, Kinnunen T, Komulainen J, Peränen N, Rönnemaa T, et al. Gestational diabetes (online). Current Care Guidelines. Working group set up by the Finnish

Medical Society Duodecim, the Medical Advisory Board of the Finnish Diabetes Association and the Finnish Gynecological Association, 2013 [Updated 25.6.2013]. Available online at: www.kaypahoito.fi.

(31) Saquib N, Stefanick ML., Natarajan L, Pierce JP. Mortality risk in former smokers with breast cancer: Pack-years vs. smoking status. Int J Cancer 2013;13310:2493-97.

(32) World Medical Association Inc. Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Indian Med Assoc 2009;1076:403-05.

(33) Vilmi-Kerälä T, Palomäki O, Kankkunen P, Juurinen L, Uotila J, Palomäki A. Oxidized LDL, insulin resistance and central blood pressure after gestational diabetes mellitus. Acta Obstet Gynecol Scand 2016;95(12):1425-32.

(34) Chenillot O, Henny J, Steinmetz J, Herbeth B, Wagner C, Siest G. High sensitivity C-reactive protein: biological variations and reference limits. Clin Chem Lab Med 2000;3810:1003-11.

(35) Sanchez A, Mirabel JL, Barrenechea E, Eugui J, Puelles A, Castaneda A. Evaluation of an improved immunoturbidimetic assay for serum C-reactive protein on a COBAS INTEGRA 400 Analyzer. Clin Lab 2002;485-6:313-17.

(36) Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 1998;9815:1487-94.

(37) Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2001;215:844-48.

(38) Monzillo LU, Hamdy O. Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev 2003;6112:397-412.

(39) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;287:412-19.

(40) Cohn JN, Finkelstein S, McVeigh G, Morgan D, LeMay L, Robinson J, et al. Noninvasive pulse wave analysis for the early detection of vascular disease. Hypertension 1995;263:503-08.

(41) Cohn JN. Vascular wall function as a risk marker for cardiovascular disease. J Hypertens Suppl 1999;175:S41-4.

(42) O'Rourke M. Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. Hypertension 1990;154:339-47.

(43)) Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;5513:1318-27.

(44) Cho Y, Lee SG, Jee SH, Kim JH. Hypertriglyceridemia is a major factor associated with elevated levels of small dense LDL cholesterol in patients with metabolic syndrome. Ann Lab Med 2015;35(6):586-94.

(45) Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, et al. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the

Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol 2014;34(5):1069-77.

(46) Lucero D, Lopez GI, Gorzalczany S, Duarte M, Gonzalez Ballerga E, Sorda J, et al. Alterations in triglyceride rich lipoproteins are related to endothelial dysfunction in metabolic syndrome. Clin Biochem 2016;49(12):932-35.

(47) Wakatsuki A; Ikenoue N; Shinohara K; Watanabe K; Fukaya T. Small low-density lipoprotein particles and endothelium-dependent vasodilation in postmenopausal women. Atherosclerosis 2004;177(2):329-36.

(48) Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol 2005;255:932-43.

(49) Prenner SB, Chirinos JA. Arterial stiffness in diabetes mellitus. Atherosclerosis 2015;2382:370-79.

(50) Tziomalos K, Athyros VG, Karagiannis A, Mikhailidis DP. Endothelial dysfunction in metabolic syndrome: prevalence, pathogenesis and management. Nutr Metab Cardiovasc Dis 2010;202:140-46.

(51) Ge JY, Li XL, Zhang HF, Xu Q, Tong M, Wang JG. Elasticity indices of large and small arteries in relation to the metabolic syndrome in Chinese. Am J Hypertens 2008;212:143-47.

(52) Pohjantähti-Maaroos H, Palomäki A, Kankkunen P, Husgafvel S, Knuth T, Vesterinen K, et al. Arterial elasticity and oxidized LDL among men with metabolic syndrome and different 10-year cardiovascular risk estimated by FINRISK and SCORE models. Ann Med 2012;445:503-12.

(53) Palomäki A, Pohjantähti-Maaroos H, Wallenius M, Kankkunen P, Aro H, Husgafvel S, et al. Effects of dietary cold-pressed turnip rapeseed oil and butter on serum lipids, oxidized LDL and arterial elasticity in men with metabolic syndrome. Lipids Health Dis 2010;9:137-511X-9-137.

(54) Pohjantähti-Maaroos H, Palomäki A, Hartikainen J. Erectile dysfunction, physical activity and metabolic syndrome: differences in markers of atherosclerosis. BMC Cardiovasc Disord 2011;11:36-2261-11-36.

(55) Cereda E, Malavazos AE, Caccialanza R, Rondanelli M, Fatati G, Barichella M. Weight cycling is associated with body weight excess and abdominal fat accumulation: a cross-sectional study. Clin Nutr 2011;306:718-23.

(56) Lehtonen HM, Suomela JP, Tahvonen R, Vaarno J, Venojärvi M, Viikari J, et al. Berry meals and risk factors associated with metabolic syndrome. Eur J Clin Nutr 2010;646:614-21.

(57) Borch-Johnsen K, Wareham N. The rise and fall of the metabolic syndrome. Diabetologia 2010;534:597-99.

(58) Simmons RK, Alberti KG, Gale EA, Colagiuri S, Tuomilehto J, Qiao Q, et al. The metabolic syndrome: useful concept or clinical tool? Report of a WHO Expert Consultation. Diabetologia 2010;534:600-05.

(59) Reaven GM. Syndrome X. Blood Press Suppl 1992;4:13-16.

(60) Cameron AJ, Zimmet PZ, Shaw JE, Alberti KG. The metabolic syndrome: in need of a a global mission statement. Diabet Med 2009;263:306-09.