Crosstalk between the sympathetic nervous system, inflammation and coagulation in gestational diabetes; a therapeutic approach in postmenopausal hypertension

Maritta Pöyhönen-Alho

Academic dissertation

To be presented by permission of the Medical Faculty of the University of Helsinki for public examination in the Auditorium 2 of Biomedicum Helsinki, Haartmaninkatu 8, 00290 Helsinki, on Friday April 29th 2011, at noon.
To my family, Mika, Isa, Ella and Aku
TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS........................................................................................................ 7

ABBREVIATIONS.................................................................................................................................... 8

ABSTRACT............................................................................................................................................... 10

FINNISH SUMMARY............................................................................................................................ 12

INTRODUCTION....................................................................................................................................... 14

REVIEW OF THE LITERATURE............................................................................................................. 17

Autonomic nervous system...................................................................................................................... 17
  Anatomy and functions........................................................................................................................ 17
  Assessment of ANS function.............................................................................................................. 18
    Plasma noradrenaline.......................................................................................................................... 18
    Orthostatic test................................................................................................................................. 20
    Baroreflex sensitivity....................................................................................................................... 20
    Hand grip.......................................................................................................................................... 20
    Noradrenaline spillover..................................................................................................................... 20
    Muscle sympathetic nervous activity.............................................................................................. 21
    Heart rate variability....................................................................................................................... 21
  Clinical implications of autonomic dysfunction................................................................................. 23

Inflammation.......................................................................................................................................... 24
  Inflammatory mediators...................................................................................................................... 25
    Adiponectin....................................................................................................................................... 25
    Alpha-1-acid glycoprotein............................................................................................................... 28
    C-reactive protein............................................................................................................................. 28
    Serum amyloid A............................................................................................................................ 29
    Sex hormone-binding globulin........................................................................................................ 29
    Tumour necrosis factor α.................................................................................................................. 30
  Inflammation and atherosclerosis...................................................................................................... 31
  Inflammation and the sympathetic nervous system........................................................................... 31

Adrenomedullin...................................................................................................................................... 32

Coagulation............................................................................................................................................ 33
  Factor VIII......................................................................................................................................... 34
  Von Willebrand Factor........................................................................................................................ 34
  Coagulation and atherosclerosis......................................................................................................... 36
  Coagulation and the sympathetic nervous system............................................................................. 36
Physiological alterations in pregnancy

- Haemodynamic changes
- Metabolic changes
  - Lipids
  - Insulin sensitivity and glucose homeostasis
- Coagulation
- Inflammation
- Sympathetic nervous system

Gestational diabetes

- Definition
- Pathophysiology
  - Insulin resistance
  - Pancreatic β-cell function
  - Genetic factors
  - Role of the placenta and inflammation
  - Modifiable factors
- Diagnosis and screening
- Epidemiology
- Long-term health concerns
  - Type 2 diabetes
  - Cardiovascular diseases
  - Health consequences of gestational diabetes in the second generation
- Prevention

Sympathetic nervous system, insulin sensitivity and inflammation after menopause

- Sympathetic nervous system
- Insulin resistance
- Inflammation
- Postmenopausal hypertension

AIMS OF THE STUDY

SUBJECTS AND METHODS

Subjects

Methods

Publications I–III

Blood sample analyses

HRV analyses

Publication IV

Statistics

Publications I–III

Publication IV
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESULTS</td>
<td>56</td>
</tr>
<tr>
<td>Characteristics of the study groups</td>
<td>56</td>
</tr>
<tr>
<td>Publications I–III</td>
<td>56</td>
</tr>
<tr>
<td>Publication IV</td>
<td>56</td>
</tr>
<tr>
<td>Sympathetic nervous system</td>
<td>58</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>58</td>
</tr>
<tr>
<td>Heart rate variability</td>
<td>59</td>
</tr>
<tr>
<td>Inflammatory markers</td>
<td>60</td>
</tr>
<tr>
<td>Insulin</td>
<td>62</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>63</td>
</tr>
<tr>
<td>Haemostatic variables</td>
<td>64</td>
</tr>
<tr>
<td>Coagulation and fibrinolysis</td>
<td>64</td>
</tr>
<tr>
<td>Platelet function</td>
<td>64</td>
</tr>
<tr>
<td>Correlation analyses</td>
<td>66</td>
</tr>
<tr>
<td>Sub-analysis of women with gestational diabetes with and without hypertension</td>
<td>67</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>68</td>
</tr>
<tr>
<td>Sympathetic nervous system</td>
<td>68</td>
</tr>
<tr>
<td>Inflammation</td>
<td>70</td>
</tr>
<tr>
<td>Coagulation</td>
<td>72</td>
</tr>
<tr>
<td>Gestational diabetes with and without hypertension</td>
<td>74</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>76</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>79</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>82</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals:


II Pöyhönen-Alho M., Ebeling P., Saarinen A., Kaaja R: Decreased variation of inflammatory markers in gestational diabetes. Diabetes Metabolism Reviews and Research, accepted DOI: 10.1002/dmrr.1170


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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGP</td>
<td>alpha-1 acid glycoprotein</td>
</tr>
<tr>
<td>AM</td>
<td>adrenomedullin</td>
</tr>
<tr>
<td>ANS</td>
<td>autonomic nervous system</td>
</tr>
<tr>
<td>APTT</td>
<td>activated partial thromboplastin time</td>
</tr>
<tr>
<td>AR</td>
<td>adrenoreceptor, catecholamine receptor</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CO/ADP</td>
<td>collagen/adenosine diphosphate-coated test cartridge for assessment of primary haemostasis</td>
</tr>
<tr>
<td>CO/EPI</td>
<td>collagen/adrenaline-coated test cartridge for assessment of primary haemostasis</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CT</td>
<td>closure time, a variable used in assessment of primary haemostasis with the PFA-100&lt;sup&gt;®&lt;/sup&gt; system</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>FVIII:C</td>
<td>coagulation factor VIII activity</td>
</tr>
<tr>
<td>GDM</td>
<td>gestational diabetes mellitus</td>
</tr>
<tr>
<td>HF</td>
<td>high-frequency oscillations in HRV analysis, 0.15–0.4 Hz</td>
</tr>
<tr>
<td>HRV</td>
<td>heart rate variability</td>
</tr>
<tr>
<td>IGF-1</td>
<td>insulin-like growth factor-1</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin-6</td>
</tr>
<tr>
<td>LF</td>
<td>low-frequency oscillations in HRV analysis, 0.04–0.15Hz</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>MSNA</td>
<td>muscle sympathetic nerve activity</td>
</tr>
<tr>
<td>NA</td>
<td>noradrenaline</td>
</tr>
</tbody>
</table>
NPCs non-pregnant controls
nu normalized units
OGTT oral glucose tolerance test
PAI-1 plasminogen activator inhibitor-1
PCs pregnant controls
PFA-100® platelet function analyzer
PNS parasympathetic nervous system
PT prothrombin time
RMANOVA repeated-measures analysis of variance
SAA serum amyloid A
SDANN standard deviation of the average normal-to-normal intervals for each 5-min period in HRV analysis
SDNN standard deviation of normal-to-normal intervals
SHBG sex hormone-binding globulin
SNS sympathetic nervous system
T2D type 2 diabetes
TF tissue factor
TNFα tumour necrosis factor α
tPA tissue plasminogen activator
TT thrombin time
ULF ultra-low frequency oscillations in HRV analysis, <0.003 Hz
VLF very-low frequency oscillations in HRV analysis, 0.003–0.04 Hz
VTE venous thromboembolism
vWF:Ag von Willebrand factor antigen, determines amount of vWF
vWF:CB von Willebrand factor collagen-binding activity
vWF:RCo von Willebrand factor ristocetin cofactor activity
ABSTRACT

The occurrence of gestational diabetes (GDM) during pregnancy is a powerful sign of a risk of later type 2 diabetes (T2D) and cardiovascular diseases (CVDs). The physiological basis for this disease progression is not yet fully understood, but increasing evidence exists on interplay of insulin resistance, subclinical inflammation, and more recently, on unbalance of the autonomic nervous system. Since the delay in development of T2D and CVD after GDM ranges from years to decades, better understanding of the pathophysiology of GDM could give us new tools for primary prevention.

The present study was aimed at investigating the role of the sympathetic nervous system (SNS) in GDM and its associations with insulin and a variety of inflammatory cytokines and coagulation and fibrinolysis markers. We hypothesized that the SNS is activated in pregnancy complicated by GDM when compared with normal pregnancy and that this activation is associated with changes in low-grade tissue inflammation and coagulation. We further hypothesized that by reducing SNS activity it would be possible to improve the inflammatory profile in women at risk.

This thesis covers two separate study lines. Firstly, we investigated 41 women with GDM and 22 healthy pregnant and 14 non-pregnant controls during the night in hospital. Blood samples were drawn at 24:00, 4:00 and 7:00 h to determine the concentrations of plasma glucose, insulin, noradrenaline (NA) and adrenomedullin, markers of subclinical inflammation (C-reactive protein, interleukin-6, insulin-like growth factor-1, serum amyloid A, sex hormone-binding globulin, acid alpha-1 glycoprotein and cortisol), coagulation and fibrinolysis variables (thrombin time, thromboplastin time, activated partial thromboplastin time, factor VIII, fibrinogen, von Willebrand factor antigen, ristocetin cofactor activity and collagen binding activity as well as D-dimer) and platelet function. Overnight holter ECG recording was performed for analysis of heart rate variability (HRV). Secondly, we studied 87 overweight hypertensive women with natural menopause. They were randomised to use a central sympatholytic agent, moxonidine (0.3mg twice daily), the β-blocking agent atenolol (50 mg once daily+placebo once daily) for 8 weeks. Inflammatory markers (C-reactive protein, tumour necrosis factor alpha [TNFα] and its receptor II, and interleukin-6) and adiponectin were analysed at the beginning and after 8 weeks.
Activation of the SNS (increase in NA, decreased HRV) was seen in pregnant vs. non-pregnant women, but no difference existed between GDM and normal pregnancy. However, modulation (internal rhythm) of HRV was attenuated in GDM. Insulin and inflammatory cytokine levels were comparable in all pregnant women but nocturnal variation of concentrations of C-reactive protein, serum amyloid A and insulin were reduced in GDM. Levels of coagulation factor VIII were lower in GDM compared with normal pregnancy, whereas no other differences were seen in coagulation and fibrinolysis markers. No significant associations were seen between NA and the studied parameters.

In the study of postmenopausal women, moxonidine treatment was associated with favourable changes in the inflammatory profile, seen as a decrease in TNFα concentrations (increase in atenolol group) and preservation of adiponectin levels (decrease in atenolol group).

In conclusion, our results did not support our hypotheses of increased SNS activity in GDM or a marked association between NA and inflammatory and coagulation markers. Reduced biological variation of HRV, insulin and inflammatory cytokines suggests disturbance of autonomic and hormonal regulatory mechanisms in GDM. This is a novel finding. Further understanding of the regulatory mechanisms could allow earlier detection of risk women and the possibility of prevention. In addition, our results support consideration of the SNS as one of the therapeutic targets in the battle against metabolic diseases, including T2D and CVD.

Tämän väitöstutkimuksen tarkoituksena oli selvittää symppaattisen hermoston toimintaa raskausdiabeteksessä sekä sen yhteyksiä sokeriaineenvaihduntaan, tulehdukseen ja hyyttymisjärjestelmään. Hypoteesimme oli, että raskausdiabetekseen liittyy symppaattisen hermoston aktivaatio, joka heijastuu tulehdus- ja hyyttymistekijöihin. Lisäksi tutkimme, voidaanko symppaattisen hermoston aktivaatiota vähentämällä vaikuttaa suotuisasti tulehdukseen naisilla, joilla on lisääntynyt sydän- ja verisuonisairauksien riski.

Väitöskirja koostuu kahdesta erillisestä tutkimuslinjasta. Ensimmäisessä tutkimuksessa 41 raskausdiabeetikolle, 22 terveelle raskaana olevalle sekä 14:lle ei-raskaana olevalle naiselle tehtiin yöllinen EKG-nauhoitus sydämen autonomisen säätelyn tutkimista varten (sykevaihtelualalyysi). Lisäksi heiltä määritettiin veren glukoosi- ja insuliinipitoisuus, noradrenaliini ja adrenomedulliini, tietty tulehdusmerkkiala, C-reacttiivinen proteiini eli CRP, interleukiini-6, insuliinikasvutekijä-1, amyloidi A, sukupuolihormoneja sitova globuliini, alfa1-glykoproteiini ja kortisoli, tietty hyyttymisaktivaation merkkiala (trombiini, tromboplastiinia, aktivoitu partiaalinen tromboplastiinia, hyyttymistekijä VIII, fibrinogeeni, von Willebrandin tekijän antigeeni, ristosetiihin aktiivisuus ja kollageenin sitomisaktiivisuus sekä D-dimeeri) ja verhiutaleaktivaatio. Toisessa tutkimuksessa 87 vahdevuosi-ikäistä verenpainetta sairastavaa ylipainoista naista satunnaistettiin saamaan kahdeksan viikon ajan joko keskushermoston kautta symppaattisen hermoston toimintaa hillitsevää verenpainelääkettä moksonidiinia (0,3 mg kahdesti päivässä) tai betasalpaajaa atenololia (50 mg kerran päivässä + lumeppiieri). Tutkimuksen alussa ja loppussa määritettiin tietty tulehdusmerkkiala (CRP, tuumorinekrositekijä alfa eli TNF-α ja sen reseptori II, interleukiini-6 ja adiponektiini).

Lääketutkimuksessamme tuli esiin moksonidiiniryhmässä suotuisa tulehdusprofiilin muutos verrattuna atenololiryhmään. Moksonidiinia saaneilla naisilla haitallinen TNF-α-pitoisuus pieneni, kun taas atenololiryhmässä pitoisuus kasvoi. Suojaavan adiponektiinin pitoisuus pysyi näillä naisilla ennallaan, mutta atenololiryhmässä tapahtui merkittävä lasku.

INTRODUCTION

Coronary artery disease (CAD) is the most common cause of death in women in the Western world. Atherosclerosis typically develops, progresses and festers for decades in a clinically silent fashion, finally manifesting itself in a life-threatening catastrophe. Because of this treacherous development, various strategies to identify individuals at risk have been suggested (Ambrose and Srikanth 2010). Recent meta-analysis and randomized trial data indicate that global CAD risk information and aggressive multimodal therapy targeting the modifiable cardiovascular risk factors might contribute to improved prevention and reduction in the occurrence of adverse cardiovascular events (Sheridan et al. 2010, Mosca et al. 2007, Yusuf et al. 2004).

The aetiopathogenesis of CAD is multifactorial, affected by lipid and lipoprotein changes, hypertension and metabolic factors, such as elevated blood glucose concentrations and various modified (oxidation, glycation) proteins (Bucala et al. 1994). Increased production of procoagulants, adhesion molecules, chemotactic factors and cytokines further contributes to this development. Even though the mechanisms behind these deleterious changes are multiple and complexly interrelated, some key elements of pathogenesis can be identified. Harmful inflammation seems to be a central denominator. Subclinical inflammation independently predicts adverse cardiovascular events and progression of coronary atherosclerosis (Nissen et al. 2004). The inflammatory system is closely related to the renin-angiotensin system (RAS) and the autonomic regulatory system, especially the sympathetic nervous system (SNS). In unfavourable conditions this crosstalk seems to create a vicious circle favouring the development of atherosclerosis. The connections demonstrated between the inflammatory system, coagulation and the autonomic nervous system are presented in Figure 1.

Type 2 diabetes (T2D) has been considered to be a CAD risk equivalent to a >20% risk of developing a new major coronary event within 10 years following diagnosis (Haffner et al. 1998). One of the most powerful early predictors of T2D is gestational diabetes mellitus (GDM), which refers to glucose intolerance with onset or first recognition during pregnancy. Up to 70% of women with GDM develop T2D within 10 years after pregnancy (Kim et al. 2002). GDM is characterized by both pronounced insulin resistance and inflammation compared with normal pregnancy (Volpe et al. 2007, Barbour et al. 2007, Challis et al. 2009, Metzger et al. 2007). Much less is known about the function of the autonomic nervous system (ANS) and coagulation in GDM.
This thesis concerns investigation of SNS activity in GDM and associations between the SNS, inflammation and coagulation. Better understanding of the pathogenesis of GDM is mandatory in the aim to build new strategies for prevention of metabolic diseases after GDM.
Figure 1. Connections demonstrated between the SNS, inflammation and coagulation. Figure modified from Elenkov et al. 2000.

NA, noradrenaline; TF, tissue factor; vWF, von Willebrand factor, FVIII, factor VIII; tPA, tissue plasminogen activator
REVIEW OF THE LITERATURE

Autonomic nervous system

The autonomic nervous system (ANS) controls (acting below the level of consciousness) visceral functions of the body. These functions include the automatism of the heart, blood pressure, respiration rate, digestion, salivation, perspiration, micturition and sexual arousal. The ANS is divided into three subsystems: the sympathetic nervous system (SNS), the parasympathetic nervous system (PNS), also called the vagal system, and the enteric nervous system, which is integral in autonomic function, particularly in the gut and the lungs.

Anatomy and functions

The function of the ANS is organized on the basis of reflex arcs comprising a visceral receptor, an afferent pathway, the central nervous system (CNS), an efferent pathway and the effector organ. Baroreceptors and chemoreceptors in the viscera monitor arterial pressure as well as the levels of multiple substances and chemicals, such as carbon dioxide, oxygen and glucose. Input from these receptors is integrated in regulation centres in the CNS for constant modulation of the activity of the efferent neurons. The efferent autonomic nerves consist of preganglionic and postganglionic neurons with synapses in autonomic ganglia which in the SNS are localized close to the spinal cord and in the PNS in or near the innervated organ. The postganglionic neurons innervate the effector organs (Figure 2). Acetylcholine is the preganglionic neurotransmitter for both the SNS and the PNS. At the effector organs, the PNS uses chiefly acetylcholine, whereas sympathetic neurons release noradrenaline (NA) to act mainly on \( \alpha \)- and \( \beta \)-adrenergic receptors (ARs) (Soinila 2006).

The SNS and the PNS typically function reciprocally. Their functions should, however, be regarded as complementary rather than antagonistic in the effort to achieve homeostasis. The SNS typically functions in actions requiring quick responses (‘fight or flight’). It is responsible for overall activation and energy generation in the body. When activated, the SNS enhances blood flow to critical organs, such as skeletal muscles (locomotion), the liver (energy supply) and the lungs (oxygen supply). When the situation lapses, the PNS promotes a return to a resting tone (‘rest and digest’). Functions of the SNS and the PNS in specific organs are summarized in Table 1.
Assessment of ANS function

Because of the complexity of the ANS, no single method exists in assessment of its functions. In addition, ANS responses typically show regional or organ-specific differentiation, with varying activities of the SNS and the PNS depending on the organ examined. Even though some tests provide data to assess the SNS specifically, the clinical outcome always reflects the balance between the SNS and PNS.

*Plasma noradrenaline*

Noradrenaline (NA) works both as a neurotransmitter in the CNS and as a hormone, released by the adrenal gland together with adrenaline. Measurement of NA concentration in venous blood represents one of the most commonly employed indexes of sympathetic activity in man. Reproducibility and sensitivity of this method in non-standardized conditions are limited, however, although reproducibility can be improved by repeated sampling (Grassi et al. 2009). In addition, discrimination between changes in secretion and/or clearance is not possible. In clinical studies and
in well-defined conditions, plasma NA levels seem to correlate relatively well with more specific SNS assessment methods (Kaaja and Pöyhönen-Alho 2006).

Table 1. Actions of the sympathetic and the parasympathetic nervous systems.

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>SYMPATHETIC</th>
<th>PARASYMPATHETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart rate</td>
<td>increases</td>
<td>decreases</td>
</tr>
<tr>
<td>contractility</td>
<td>increases</td>
<td>decreases</td>
</tr>
<tr>
<td><strong>Blood vessels: arteries</strong></td>
<td>constricts</td>
<td>no effect</td>
</tr>
<tr>
<td>kidneys, viscera, skin, brain coronaries, larger coronaries, smaller, salivary gland, erectile tissue liver, skeletal muscle veins</td>
<td>constricts/dilates</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>Bronchioles</strong></td>
<td>relaxes (major contribution) constricts (minor contribution)</td>
<td>constricts</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td>glycogenolysis, gluconeogenesis</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td>renin secretion</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>GI tract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>motility</td>
<td>decreases</td>
<td>increases</td>
</tr>
<tr>
<td>sphincters</td>
<td>contract</td>
<td>dilate</td>
</tr>
<tr>
<td>secretion</td>
<td>no effect</td>
<td>increases</td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucagon secretion</td>
<td>increases</td>
<td>increases</td>
</tr>
<tr>
<td>insulin secretion</td>
<td>decreases</td>
<td>increases</td>
</tr>
<tr>
<td><strong>Adrenal medulla</strong></td>
<td>noradrenaline and adrenaline secretion</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>aggregation</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>Eye</strong></td>
<td>mydriasis</td>
<td>miosis</td>
</tr>
<tr>
<td>long-range focus</td>
<td></td>
<td>short-range focus</td>
</tr>
<tr>
<td><strong>Urinary system</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>detrusor muscle</td>
<td>dilates</td>
<td>contracts</td>
</tr>
<tr>
<td>urethral sphincter</td>
<td>contracts</td>
<td>dilates</td>
</tr>
<tr>
<td><strong>Reproductive system</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uterus</td>
<td>contracts (pregnant), dilates (non-pregnant)</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>Sweat gland</strong></td>
<td>secretion</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>Adipose tissue</strong></td>
<td>lipolysis</td>
<td>no effect</td>
</tr>
</tbody>
</table>
Orthostatic test

The posture change from lying to upright is known as the orthostatic test. The heart rate accelerates and blood pressure decreases after a change to a standing position. Blood pressure and heart rate differences between lying and upright postures in the orthostatic test relate to blood norepinephrine levels, and the orthostatic test has been used in non-invasive evaluation of the SNS (Ewing et al. 1985).

Baroreflex sensitivity

Baroreflex sensitivity (BRS) means the effect of a change in blood pressure on the ensuing heart period. An increase in blood pressure is followed by increased heart beat duration and the steepness of the linear correlation between changes of systolic pressure and heart beat duration (ms/mmHg) gives an indication of BRS (Smyth et al. 1969). For assessing BRS, measurement of ECG, blood pressure, and optionally respiration, are analyzed by using various computerized methods. BRS reflects both PNS and SNS activity, and the information concerning the SNS has not been shown to be consistent (Karemaker 2002).

Hand grip

Static handgrip brings about marked increases in heart rate, arterial pressure, and muscle sympathetic nerve activity (Mark et al. 1985, Khurana and Setty 1996). Static handgrip is performed with the dominant hand, usually at 30% of maximal voluntary contraction, for 5 minutes. Changes in heart rate and blood pressure are monitored (Khurana and Setty 1996).

NA spillover

Plasma NA concentrations depend on sympathetic tone-induced NA secretion as well as removal (clearance) of the neurotransmitter from plasma. ‘Net’ NA secretion can be studied by measuring the appearance of radio-labelled NA into specific organs during intravenous infusion. Using NA plasma kinetic methodology, total body noradrenaline spillover to plasma can also be calculated (Esler et al. 1979). This dynamic process of NA release and removal has been shown to quantify sympathetic nervous activity better than steady-state plasma NA measurement alone (Esler et al. 1979, Esler et al. 1988). Use of radioactive tracers, invasiveness of the method and need for special laboratory circumstances limits the use of NA spillover in clinical settings. During pregnancy, use of this method is contraindicated.
**Muscle sympathetic nervous activity**

Investigation of muscle sympathetic nerve activity (MSNA), assessed by microneurography, represents the only method available for direct recording of sympathetic nerve activity. Thin electrodes (10 μm) are inserted into a single nerve fibre of a skeletal (usually peroneal) muscle to record muscle sympathetic nerve impulses. Assessment of MSNA is an on-line dynamic method highly reproducible in healthy subjects (Vallbo et al. 1979). However, it is invasive and its use is restricted to the special laboratory environment.

**Heart rate variability**

Heart rate and rhythm are largely under the control of the ANS (Jalife et al. 1983). They are considered to result from sympatho-vagal interaction on the sinus node and to represent cardiac autonomic regulation (Ewing et al. 1984). Within the ANS, various regulatory systems associated with respiration, vasomotor control, baroreceptor reflexes and thermoregulation affect heart rate variability (HRV) (Malliani et al. 1991).

Heart rate variability reflects fluctuations in the duration of consecutive R-R intervals (intervals between adjacent QRS complexes resulting in sinus node depolarization). It is influenced by respiration and it can be represented by the ratio of the duration of the longest heart beat in expiration divided by the duration of the shortest heart beat in inspiration, or the difference between respective heart beat durations. As analysis of HRV is based on mathematical models, it is relatively easily accomplished by using modern technology. In HRV analysis, the original ECG signal is converted from analogue to digital in a microcomputer. Before analyzing, the original ECG data must be edited by visual inspection and processing of artifacts and ectopic beats, which can markedly interfere with the analysis. The time intervals between consecutive peaks of the R waves form a tachogram (Figure 3).

HRV can be analyzed as a function of time (time domain) and/or according to the distribution of variance during recording (frequency domain). In the time domain measures, the standard deviation of R-R intervals (SDNN) reflects the overall HRV in the period of recording. SDNN varies depending on the length of the recording period and therefore only recordings of same duration are appropriate for comparison (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Other commonly used time domain measures include estimated changes in the long-term or short-term components of HRV. In
frequency domain analysis, also called power spectral density analysis, HRV is described as the sum of elementary oscillatory components defined by their frequency and amplitude (Figure 3) (Akselrod et al. 1981). Various algorithms can be used to assess the number, frequency and amplitude of the oscillatory components. Fast Fourier Transform (FFT, non-parametric) or autoregressive (parametric) approaches are most commonly used.

Four major frequency bands are distinguished in a power spectrum from healthy subjects: high frequency (HF, 0.15–0.4 Hz), low frequency (LF, 0.04–0.15 Hz), very low frequency (VLF, 0.003–0.04 Hz) and ultra-low frequency (ULF, <0.003 Hz) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). The periodicities of fluctuation detected by these components are 2.5–7 s, 7–25 s, 25 s–6 min and >6
The powers of individual frequency components are represented by the areas under the proportion of the curve related to each component, and expressed both in absolute units (ms²) and normalized units (nu). Normalization minimizes the effect of changes in total power on the values of the HF and LF components. Normalized units are obtained by dividing the power of the given component by the total variance from which the VLF and ULF components have been subtracted and then multiplying by 100 (Malliani et al. 1991). The HF and LF components account for only approximately 5% of the total power, leaving 95% to ULF and VLF power. The physiological correlates of ULF and VLF are largely unknown.

HRV represents mainly the activity of the PNS and the impact of the SNS on HRV is not clear. Decreased HRV (a.k.a. SDNN) is agreed to represent increased sympathetic activation, which can result, however, from both parasympathetic withdrawal and/or increased sympathetic input (Akselrod et al. 1985, Pomeranz et al. 1985, Pagani et al. 1986, Taylor et al. 1998, Malliani et al. 1991, Eckberg 1997).

In clinical studies, changes of HRV related to specific pathologies have been convincingly demonstrated only in the time domain parameters. In addition, the practical use of (depressed) HRV is clearly shown only as a predictor of risk for MI recurrence and as an early warning sign of diabetic neuropathy (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996).

**Clinical implications of autonomic dysfunction**

As the ANS regulates various vital mechanisms of the human body, disturbances in its function may lead to detrimental events. Autonomic imbalance has been suggested as the final common pathway to increased morbidity and mortality from a host of conditions and diseases (Thayer et al. 2010). Changes in HRV have been found to be significantly associated with all-cause mortality after controlling for other risk factors (Tsuji et al. 1996, Liao et al. 2002).

Heart failure, unstable angina and acute MI are associated with striking sympathetic overactivity, and extreme sympathoexcitation predicts mortality in patients with these conditions (Kleiger et al. 1987, Odemuyiwa et al. 1991). Low HRV is also associated with the pathophysiology of CVD, i.e. progression of atherosclerosis (Huikuri et al. 1999), evolution of myocardial infarction (Tsuji et al. 1996) and onset of arterial hypertension (Singh et al. 1998). In addition, increased SNS activity is associated with co-morbidities of CVD, such as essential hypertension (Fagard et al. 2001, Lucini et
al. 2002), obesity (Scherrer et al. 1994, Grassi et al. 1995, Sztajzel et al. 2009) and obstructive sleep apnoea (Narkiewitz et al 1998). The association between depressive disorders and sympathetic overactivity was demonstrated decades ago (Esler et al. 1982), but only recently brought up in relation to increased coronary heart disease risk in such patients (Esler 2009).

Cardiovascular autonomic neuropathy is a general complication of diabetes characterized by widespread neuronal degeneration of small nerve fibres of both the sympathetic and parasympathetic tracts (Töyry et al. 1996, Duby et al. 2004). It is a key cause of morbidity and mortality in diabetes. The disease process may begin early in the course of diabetes but remain asymptomatic until later stages. HRV analysis have been proven to be useful in detecting diabetes-associated neuropathy, thus allowing risk stratification and earlier planning of subsequent management (Smith 1982, Ewing et al. 1991, Malpas and Maling 1990, Bellavere et al. 1992, Cacciatori et al. 1997, Schonauer et al. 2008).

Inhibition of SNS activity is an effective means of lowering blood pressure. Peripheral α- and β-AR blocking agents belong to the conventional drugs in treatment of hypertension. In contrast to peripherally acting agents, central sympatholytic agents have the advantage of preserving normal physiological activation of the SNS during postural adjustments and exercise (Ernsberg et al. 1997, Greenwood et al. 2000). Such centrally acting SNS inhibiting agents include clonidine and newer antihypertensive drugs, imidazoline-1 receptor-specific agents, such as moxonidine and rilmenidine. Sympatholysis-mediated antihypertensive effects of these agents have been well documented. They reduce plasma catecholamine levels and muscle sympathetic activity as well as plasma renin in hypertension (Wenzel et al. 1998, Greenwood et al. 2000, Hausberg et al. 2010). There is also evidence of an effect of moxonidine on HRV (De Vito et al. 2002, Kaya et al. 2010). Besides their antihypertensive effects, improvement in glucose tolerance has been suggested in some, but not all studies (Ernsberg et al. 1997, Penicaud et al. 1998, Haenni and Lithell 1999, Masajtis-Zagajewska et al. 2010).

**Inflammation**

Inflammation represents a physiological protective process to maintain tissue homeostasis in response to harmful stimuli. Both exaggerated and insufficient inflammatory responses may lead to
a variety of diseases. Inflammation may be caused by infection, but the inflammatory process may also be triggered by non-pathogens, such as damaged cells. Low-grade tissue inflammation refers to non-pathogen-induced inflammation with long-term low levels of inflammatory markers found either in the circulation or in tissues.

Acute-phase proteins (APPs) are a class of proteins whose plasma concentrations increase or decrease in response to inflammation. In response to injury, local inflammatory cells (neutrophilic granulocytes and macrophages) secrete a number of cytokines, most notable of which are the interleukins (ILs) and TNFα. Liver hepatocytes, and to a lesser extent other cell types (monocytes, endothelial cells, adipocytes, fibroblasts) respond by producing a large number of APPs. APPs have a wide range of activities contributing to host defence, including inflammation-neutralizing and local tissue damage-minimizing actions as well as participation in tissue repair and regeneration.

Inflammatory mediators

Characteristics of the inflammatory mediators assessed in this study are presented in Table 2.

Adiponectin

Adiponectin, synthesized almost exclusively by adipocytes, is present in the blood at high levels compared with many other hormones (Ouchi et al. 2011). Adiponectin levels are decreased in obese compared with lean individuals in such a way that it is expressed at the highest levels by the functional adipocytes that are found in lean organisms, but its expression is down-regulated in the dysfunctional adipocytes that are associated with obesity (Ryo et al 2004).

Plenty of data indicate that adiponectin is protective against the development of obesity-linked heart diseases, and it is considered as a molecular link between adipose and cardiovascular tissues (Ouchi et al 2011). The ability of adiponectin to suppress pro-inflammatory cytokine production, at least partly by modulation of macrophage function and phenotype, may be a key mechanism (Ouchi et al. 2011). Negative correlations between adiponectin and many cytokines, such as CRP, IL-6 and TNFα, have been shown (Ouchi et al.2003). The results of several in vitro and in vivo studies have emphasized the ability of adiponectin to regulate glucose metabolism and insulin sensitivity in muscle and liver, and it has a modulatory function in vascular remodelling. Adiponectin has been
Table 2. Characteristics of the inflammatory markers investigated in this study.

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Site of production</th>
<th>Normal plasma concentration</th>
<th>Change during normal pregnancy</th>
<th>Biological functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adiponectin</strong></td>
<td>adipose tissue</td>
<td>3–30 μg/ml</td>
<td>decreases</td>
<td>increases insulin sensitivity, anti-inflammatory effect, anti-atherogenic properties</td>
<td>Diez and Iglesias 2003, Catalano et al. 2006</td>
</tr>
<tr>
<td><strong>AGP</strong></td>
<td>liver (primary) other tissues</td>
<td>0.6–1.2 mg/ml</td>
<td>decreases</td>
<td>carrier of basic drugs, steroids and protease inhibitors, anti-inflammatory and immunomodulating effects</td>
<td>Fourier et al. 2000, Paradisi et al. 2009</td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td>liver</td>
<td>0.1–3 mg/l</td>
<td>increases</td>
<td>mediator of immune responses against various pathogens and damaged host cells</td>
<td>Casas et al. 2008, Kristensen et al. 2009</td>
</tr>
<tr>
<td><strong>IGF-1</strong></td>
<td>liver (primary) other tissues</td>
<td>large variation depending on age</td>
<td>increases</td>
<td>stimulation of cell growth and proliferation, inhibition of programmed cell death</td>
<td>Le Roith 1997, Bhaumick et al. 1986</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>several cell types</td>
<td>&lt;5.9 ng/l</td>
<td>increases</td>
<td>anti-inflammatory effects, pro-inflammatory effects</td>
<td>Kamimura et al. 2003, Opsjon et al. 1993, <a href="http://www.huslab.fi">www.huslab.fi</a></td>
</tr>
</tbody>
</table>
Table 2. Characteristics of the inflammatory markers investigated in this study.

<table>
<thead>
<tr>
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<th>Change during normal pregnancy</th>
<th>Biological functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα-R II</td>
<td>immune cells endothelial cells</td>
<td>unknown</td>
<td>increases</td>
<td>receptor for TNFα, cell signalling</td>
<td>Locksley et al. 2001, Chemyshov et al. 2005</td>
</tr>
</tbody>
</table>
shown to promote vascular homeostasis and suppress the development of atherosclerosis (Ouchi et al 2003).

Circulating concentrations of adiponectin seem to decrease slightly during pregnancy (Paradisi et al. 2010) and GDM is associated with lower adiponectin concentrations than in normal pregnancy (Williams et al. 2003, Lain et al. 2008). Plasma levels of adiponectin are lower in patients with diabetes compared with non-diabetic controls, and in patients with CAD compared with age- and BMI-adjusted healthy individuals (Hotta et al. 2000, Ouchi et al. 1999). Adiponectin has shown to be an independent risk factor as regards CAD (Kumada et al. 2003).

**Alpha-1-acid glycoprotein**

The exact biological function of alpha-1-acid glycoprotein (AGP), also known as orosomucoid, is unknown. However, numerous activities of potential physiological significance have been described, including various immunomodulating effects and the ability to bind basic drugs and many other molecules, such as steroid hormones. In addition, AGP is considered as an acute-phase protein, since its serum concentrations increase several-fold during acute-phase reactions (Fournier et al. 2000). Elevated levels of AGP have been associated with cardiovascular diseases and type 2 diabetes (Duncan et al. 2003, Engström et al. 2004). No data is available on the behaviour of AGP in GDM.

**C-reactive protein**

C-reactive protein (CRP) is a classical acute-phase protein produced mainly by the liver in response to pro-inflammatory cytokines including IL-6, IL-1 and TNFα. CRP is a mediator of immune responses against various pathogens and damaged cells of the host (Casas et al. 2008). However, in a recent critical review it was stated that “Despite many claims and assertions in the literature, neither the normal functions of human CRP nor its possible role in disease is known” (Casas et al. 2008). The speed and dynamic range of CRP are remarkable, since plasma concentrations of CRP can rise by over 1000-fold in 24–48 h after a strong acute stimulus (e.g. sepsis, MI) and fall rapidly when the stimulus is removed (Casas et al. 2008).

Modestly elevated baseline CRP levels have been associated with a long-term risk of coronary heart disease in general populations and use of CRP as part of a global coronary risk assessment strategy in adults without known cardiovascular disease has been suggested (Pearson et al 2004, Danesh et
al. 2004). However, the pathogenic and clinical significance of associations between CRP and CVD is still controversial. In the context of other than acute-phase responses, the term ‘high-sensitivity’ CRP is used, and it refers to measurement of CRP using immunoassay methods with sufficient sensitivity to quantify CRP throughout its normal range (in contrast to measurement of acute phase responses).

Circulating concentrations of CRP have been shown to be higher in healthy pregnant than in healthy non-pregnant individuals (Watts et al. 1991). In GDM, CRP levels have been shown to be elevated as early as in the first trimester in women who subsequently develop the condition. However, this association seems to be at least partly explained by BMI and weight gain during pregnancy (Leipold et al. 2005, Wolf et al. 2003)

**Serum Amyloid A**

Serum amyloid A (SAA) is a member of a family of apolipoproteins of which the most responsive to inflammatory stimuli, ‘acute-phase’ SAA, discussed here, is the archetypal vertebrate major acute-phase protein. It is induced from resting levels by more than 1000-fold during inflammation, implying an important (beneficial) role in host defence (Uhlar and Whitehead, 1999).

Even though the primary physiological role of SAA remains obscure, many potential functions in humans have been proposed. These include modulation of the inflammatory response via both anti- and proinflammatory activities as well as involvement in cholesterol transport and metabolism (Uhlar and Whitehead, 1999, Urieli-Shoval et al. 2000).

An increasing amount of data suggests that SAA, through these functions, may be a direct mediator in the development and progression of atherogenesis and atherothrombosis (Hua et al. 2009). SAA has been shown to be associated with an increased risk of cardiovascular events (Hua et al. 2009) as well as type 2 diabetes (Du et al. 2009). No data is available on SAA in GDM.

**Sex hormone-binding globulin**

Sex Hormone-Binding Globulin (SHBG) is a multifunctional protein that modulates androgen and oestrogen actions in humans in two ways. By binding to plasma estradiol and testosterone, SHBG regulates the availability of sex hormones to responsive tissues. Secondly, sex hormone-bound SHBG may directly mediate cell-surface signalling and the biological actions of sex hormones
(Nakhla et al. 2009). Hyperoestrogenic states, such as pregnancy, are associated with high levels of SHBG whereas hyperandrogenism results into low levels of circulating SHBG.

Clinical studies have revealed associations between low circulating levels of SHBG and many risk factors of CVD (Sutton-Tyrrell et al. 2005), and levels of sex hormones, both free and bound fractions, show associations with the risk of T2D (Ding et al. 2006, Ding et al. 2007). In GDM and in women who develop GDM later in pregnancy, relatively low levels of SHBG have been shown as early as in the first trimester of pregnancy (Batrha et al. 2000, Nanda et al. 2011)

_Tumour necrosis factor α_

Tumour necrosis factor α (TNFα), also known as cachectin or simply TNF, was first identified as a factor causing fever and wasting. It was found to be product of lymphocytes and macrophages and cause lysis of certain types of cells, especially tumour cells (Beutler and Cerami 1986). By way of genetic studies it became evident that it is one product of a gene superfamily associated with host defence, inflammation, apoptosis, autoimmunity and organogenesis (Locksley et al. 2001).

Most organs of the body appear to be affected by TNFα and it is produced by several types of cells, but especially by macrophages. TNFα has a variety of functions, including growth-stimulating properties and growth-inhibitory processes, beneficial immune responses to bacterial and certain fungal, viral, and parasitic invasions, as well as a role in local inflammatory immune responses (Locksley et al. 2001).

Experimental data suggests that TNFα is involved in the pathogenesis of atherosclerosis by impairing endothelial function and endothelium–blood cell interaction. In addition, TNFα has been shown to impair insulin signalling (Kleinbongard et al. 2010). Increased plasma TNFα has been shown to predict a risk of cardiovascular diseases (Libby et al. 2002). In gene studies, TNFα-promoting polymorphisms have been associated with type 2 diabetes as well as with conversion of glucose intolerance to type 2 diabetes (Heijmans et al. 2002, Kubaszek 2004).

Circulating levels of TNFα have been found to increase during normal pregnancy, to correlate inversely with insulin secretion and to be associated independently with insulin sensitivity (Kirwan et al. 2002, McLachlan et al. 2006). In GDM, levels of TNFα have been found to be higher than in normal pregnancy (McLachlan et al. 2006).
Inflammation and atherosclerosis

In the pathogenesis of atherosclerosis, inflammation plays a fundamental role at all stages from inception and development to the ultimate endpoint, thrombotic complication (Libby 2002, Abela 2010). Elevated levels of several inflammatory mediators among patients with CVDs, as well as apparently healthy men and women, have proven to predict future adverse vascular events and deaths. Such mediators include CRP, IL-6, TNFα, fibrinogen and SAA (Haverkate et al. 1997, Toss et al. 1997, Harris et al. 1999, Ridker et al. 2000, Danesh et al. 2000, Libby 2002, Danesh et al. 2005).

Inflammation also links diabetes to atherosclerosis. Hyperglycaemia associated with diabetes can lead to glycation of various macromolecules, which augment the production of proinflammatory cytokines and promote other inflammatory pathways in vascular endothelial cells (Schmidt et al. 1999). Circulating concentrations of CRP and IL-6 have been shown to predict development of T2D even among individuals with no current evidence of insulin resistance (Pradhan et al. 2001).

Inflammation and the sympathetic nervous system

There is increasing evidence that the immune system and the CNS interact, via autonomic pathways, continuously modulating each other (Elenkov et al. 2000, Tracey 2002). Sympathetic nerve terminals innervate both the primary and secondary lymphoid organs, which release NA, influencing lymphocyte traffic and proliferation, cytokine production and activity of lymphoid cells (Elenkov et al. 2000, Figure 1). Activation of the SNS exacerbates local as well as general immune and proinflammatory mediator responses (Flierl et al. 2007, Flierl et al. 2009, Johnson et al. 2005). In addition, the release of catecholamines from presynaptic sympathetic nerve terminals leads to localized vasoconstriction, preventing invading pathogens from becoming systemic (Elenkov et al. 2000). On the other hand, the results of several studies indicate an inhibitory effect of the SNS on inflammatory responses by way of a decrease in the activity of natural killer cells and T cell immunity (Madden et al. 1989, van der Poll et al. 1994, Elenkov et al. 1996, Maestroni and Mazzola 2003). Cytokines can activate hypothalamic-pituitary release of glucocorticoids, which, in turn, suppress further cytokine synthesis. In addition, cells of the immune system can produce neuropeptides, such as acetylcholine (Tracey 2002).
Therapeutic agents acting in sympathetic α- and β-ARs have been shown to affect cytokine production. For example, α-AR antagonists have been shown to have inhibitory and α-AR agonists potentiating effects on TNFα production (Elenkov et al. 1996, Szelenyi et al. 2000).

**Adrenomedullin**

Human adrenomedullin (AM) is a ubiquitous hormone produced by a great number of different cell types in all tissues of the body, with the possible exception of the thyroid and thymus, and it has been shown to exhibit multifunctional biological activities in physiological as well as pathophysiological conditions (Hinson et al. 2000). Normal plasma concentrations of AM are in the range of 1 to 10 pmol/l (Hinson et al. 2000). Pregnancy is associated with a 5-fold increase in circulating AM concentrations at term and they revert to normal within 4 days after delivery (Wilson et al. 2004).

Various biological actions have been demonstrated by way of peripheral and central administration of AM in animal and human studies. These are presented in Table 3. However, the only clinical situation in which concentrations of AM are shown to increase to levels that are required for activation of its receptors, is septic shock (Hinson et al. 2000). Therefore, the clinical relevance of AM outside life-threatening infections remains unclear.

Concentrations of AM increase significantly in a number of disorders including cardiovascular diseases and diabetes (Hinson et al. 2000). Levels of AM are elevated in patients with T2D compared with healthy subjects and a stepwise increase occurs with advancing disease from impaired fasting glucose to T2D and diabetic nephropathy (Lim et al. 2007). Based on data so far, it appears that the observed changes in AM levels are compensatory to cardiovascular changes or injury, and that they represent a cardiovascular protective action (Hinson et al. 2000). However, fundamental information concerning the role of AM in humans is still lacking. Both animal and human studies have suggested an association between AM and the SNS (Taylor and Samson 2001, Krzeminski et al. 2006 and 2009, Troughton et al. 2001, Lainchbury et al. 1999).
Table 3. Biological actions of adrenomedullin.

<table>
<thead>
<tr>
<th></th>
<th>Peripheral action</th>
<th>Central action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular system</td>
<td>Vasodilatation</td>
<td>Inotrophic and chronotropic effects</td>
</tr>
<tr>
<td>Endocrine system</td>
<td>Inhibitory action on pancreatic islets</td>
<td>Inhibition of ACTH release</td>
</tr>
<tr>
<td></td>
<td>Stimulation of renin release</td>
<td></td>
</tr>
<tr>
<td>Water and fluid balance</td>
<td>Natriuresis and diuresis</td>
<td>Inhibition of water drinking and salt appetite</td>
</tr>
<tr>
<td>Autonomic nervous system</td>
<td>Desensitization of baroreflex</td>
<td>SNS stimulation Augmentation of baroreflex</td>
</tr>
<tr>
<td>Immune system</td>
<td>Antimicrobial properties</td>
<td>Anti-inflammatory effect</td>
</tr>
<tr>
<td>Coagulation (endothelial cells)</td>
<td>Attenuation of TF expression</td>
<td>Induction of release of antithrombin</td>
</tr>
<tr>
<td></td>
<td>Augmentation of synthesis and release of TFPI</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Inhibition of gastric emptying</td>
<td>Cell growth and differentiation</td>
</tr>
<tr>
<td></td>
<td>Inhibition of bronchoconstriction</td>
<td></td>
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</table>

**Coagulation**

Haemostasis is the body’s first line of defence against uncontrolled haemorrhage. Coagulation involves a complex set of protease reactions with roughly 30 different proteins (Colman et al. 2006). The coagulation cascade, shown in Figure 4, is triggered when injury to a blood vessel allows blood to come into contact with tissue factor (TF)-bearing cells. Factor Xa (FXa) is the
primary site of amplification in the process: one molecule of FXa catalyses the formation of approximately 1000 thrombin molecules (Mann et al. 2003). Activated haemostatic factor VIII (FVIII) plays a central role in the propagation of fibrin formation by activating FX in the presence of activated factor IX. The final step in the series of protease reactions leads to clot formation by transformation of soluble fibrinogen into insoluble fibrin strands forming a fibrin mesh, which ties cellular components of the clot (platelets and/or red blood cells). As soon as the clot has been formed, clot dissolution, fibrinolysis, starts to restore the structural integrity of the blood vessel wall.

**Factor VIII**

Factor VIII (FVIII) is an essential blood-clotting factor also known as anti-haemophilic factor. A genetic defect affecting FVIII results in haemophilia A, recessive X-linked coagulation disorder (Antonarakis 1995). FVIII is synthesized by vascular, glomerular and tubular endothelium, and by the liver. In the blood, it is mainly bound to vWF to form a stable complex. Upon activation by thrombin, it dissociates from the complex to interact with Factor IXa in the coagulation cascade. No longer protected by vWF, activated FVIII is proteolytically inactivated and quickly cleared from the blood stream (Lenting et al. 1998).

Elevated plasma levels of FVIII have been associated with an increased risk of venous thrombosis and pulmonary embolism (Kyrle et al. 2000). During normal pregnancy, FVIII levels seem to increase progressively by approximately 30%, beginning from the second trimester until the first 3 days after delivery (Sanchez-Luceros et al. 2003). The risk of FVIII has been assessed only in one study. Scholtes and co-workers found an increased FVIII antigen/activity ratio in GDM, but only after 32 weeks of pregnancy (Choltes et al. 1983).

**Von Willebrand Factor**

Von Willebrand factor (vWF) is a blood glycoprotein essential for normal haemostasis, and deficiency of vWF causes von Willebrand disease, the most common inherited bleeding disorder. VWF mediates the adhesion of platelets to sites of vascular damage by binding to both platelets and exposed connective tissue. As mentioned above, vWF is also required for normal factor VIII survival in the circulation (Sadler et al. 1998).
Figure 4. The coagulation cascade. Inhibitory factors in red.

**Intrinsic pathway**
- Surface contact
  - factor XII → XIIa
  - factor XI → factor XIa
  - factor IX → factor IXa
  - factor V → factor Va
  - prothrombin → thrombin
  - activated protein C
  - protein S
  - protein C + thrombomodulin

**Extrinsic pathway**
- Tissue factor → cellular injury
  - factor VII → factor VIIa
  - factor X → factor Xa
  - factor V → factor Va
  - factor IX → factor IXa
  - factor VIII → factor VIIIa
  - TFPI

**THROMBUS**
- plasmin → plasminogen
- tPA → PAI-1 and 2
- fibrin → fibrin degradation products
- fibrinogen
Von Willebrand factor has been demonstrated to be significantly predictive as regards adverse cardiac events in patients with pre-existing vascular disease, although this association is minor in the general population (Spiel et al. 2008). During normal pregnancy, levels of vWF have been shown to increase by approximately one third from the first trimester to puerperium, returning to non-pregnant levels within 2–3 weeks after delivery (Sanchez-Luceros et al. 2003). No data is available on vWF in GDM.

Coagulation and atherosclerosis

The ultimate endpoint in clinical vascular events is formation of a clot in a tapered vessel. The initiation of coagulation is triggered by TF, and platelet activation plays an important role. Elevated TF expression has been shown in all stages of atherosclerotic lesions, whereas a very low basal level in normal vessels is maintained (Wilcox et al. 1989, Steffel et al. 2006, Gertow et al. 2009). Platelet hyper-reactivity, high concentrations of many platelet-derived molecules, in particular FVIII, vWF antigen, and fibrinogen, as well as impaired fibrinolysis have been shown to be associated with and to predict atherothrombotic and venous thrombotic events (Peters et al. 1989, Cortellaro et al. 1992, Meade et al. 1993, Koster et al. 1995, Toss et al. 1997, Cushman et al. 1999, Smith et al. 2005). In addition to direct effects on the endothelium, platelets act as a ‘bridge’ for other cells within the vascular system, including leukocytes (Franks et al. 2010), thus providing a link between the coagulation and inflammatory systems. Specific interactions have been shown between the SNS and TF, macrophages (Libby and Simon 2001) and SAA (Zhao et al. 2007) (Figure 1).

Coagulation and the sympathetic nervous system

Dose-dependent stimulation of FVIII activity, vWF antigen, tissue plasminogen activator and platelets has been shown to occur shortly after an increase in the levels of circulating adrenaline. However, these responses are lacking or much weaker with NA (von Känel and Dimsdale 2000). The precise mechanisms underlying haemostatic changes in connection with sympathetic activation remain unclear. In healthy individuals, simultaneous activation of the coagulation and fibrinolysis systems by the SNS may be harmless, since the haemostatic balance is maintained within thrombosis and haemorrhage. However, in patients with impaired endothelial function, procoagulant responses appear to outweigh endothelial thromboprotective mechanisms (von Känel et al. 2001). Special attention has been paid to the early morning surge in catecholamine levels as a
result of circadian variation, possible sleep apnoea and postural change with getting up in relation to increased morning frequencies of thrombotic vascular events (von Känel and Dimsdale 2000, Masoud et al. 2008).

**Physiological alterations in pregnancy**

Pregnancy is associated with remarkable physiological alterations to ensure the survival and growth of the offspring. These changes, which are largely secondary to the effects of progesterone and oestrogen, begin after fertilization and are progressive. In the first 12 weeks of pregnancy, progesterone and oestrogen are produced predominately by the ovary and thereafter by the placenta (Ciliberto and Marx 1998). Maternal hormonal changes result in adjustments in physiology, especially in the cardiovascular, haemodynamic, haematological and metabolic systems, and lead to hypervolaemic, insulin resistant, thrombophilic and immunotolerant states. Thus, pregnancy can be regarded as a time period of general physiological stress in a woman’s life (Williams 2003, Kaaja and Greer 2005). Physiological alterations in pregnancy are summarized in Table 3.

**Haemodynamic changes**

A number of mechanisms have been postulated for hypervolaemia of pregnancy. Oestrogen increases renin levels and causes sodium retention thereby increasing total body water. In addition, other hormones with increased concentrations during pregnancy, such as prolactin, placental lactogen, prostaglandins and growth hormone, may contribute to fluid retention (Ciliberto and Marx 1998, Gallery 1998). Plasma volume increases by 40–50%, and as this increase is relatively greater than the increase in red cell mass, maternal haemoglobin concentrations fall (Bernstein et al. 2001). The increased circulating volume offers protection for the mother and foetus from the effects of haemorrhage at delivery.

Peripheral vascular resistance is reduced by 20% by oestrogen- and progesterone-mediated relaxation of vascular smooth muscle. This drop in systemic vascular resistance is a major maternal physiological adjustment to pregnancy beginning in early pregnancy, reaching a maximum in mid-pregnancy, followed by a slow rise until term (Silversides and Colman 1998). The rise in cardiac
output is facilitated by anatomical changes, namely left ventricular hypertrophy and dilatation (Silversides and Colman 1998). Renal vasodilatation and increase in glomerular filtration rate due to enhanced renal plasma flow result in increases in urea, creatinine and urate clearance. Plasma concentrations of these renal parameters are thus lower in pregnant than in non-pregnant women (Jeyabalan et al. 2003, Conrad et al. 2007).

Table 4. Physiological alterations in pregnancy.

| Haemodynamic changes                  | Increased blood volume  
|                                      | Increased heart rate  
|                                      | Increased cardiac output  
|                                      | Vasodilatation  
|                                      | Decreased blood pressure  
| Metabolic changes                     | Body fat accumulation (early pregnancy)  
| lipids                                | Catabolism (late pregnancy)  
| glucose and insulin                   | Increased triglycerides, fatty acids, cholesterol, lipoproteins and phospholipids  
|                                      | Increased insulin secretion  
|                                      | Decreased insulin sensitivity  
|                                      | Increased hepatic glucose production  
|                                      | Increased carbohydrate use  
| Haemostasis                           | Increased coagulation  
|                                      | Decreased fibrinolytic activity  
| Inflammatory system                   | Activation of the innate immune system  
|                                      | Shift from cell-mediated to humoral immunity  
| Sympathetic nervous system            | Activation (3rd trimester)  

Metabolic changes

**Lipids**

Changes in carbohydrate and lipid metabolism occur during pregnancy to ensure a continuous supply of nutrients to the growing foetus despite scanty or intermittent maternal food intake. During early pregnancy there is an increase in body fat accumulation (anabolic state), whereas late pregnancy is associated with accelerated breakdown of fat depots (catabolic state). This catabolism in late pregnancy coincides with the maximal foetal growth phase, when the mother needs to progressively increase the energy supply to the foetus (Herrera 2002). From the beginning of the 3rd trimester there are steady increases in triglycerides, fatty acids, cholesterol (total, HDL and LDL), lipoproteins and phospholipids. High oestrogen concentrations and insulin resistance are thought to be responsible for these changes (Butte 2000).

**Insulin sensitivity and glucose homeostasis**

Insulin is essential in the utilization of blood glucose by binding to specific receptors on the surface of cells which display insulin-mediated glucose uptake. Tissue responsiveness to insulin, meaning how successfully the receptor operates to permit glucose clearance, is termed insulin sensitivity. Sensitivity to insulin varies approximately three-fold in non-pregnant lean subjects with normal glucose tolerance (Davidson 1995). Abnormally low insulin sensitivity is called insulin resistance. Insulin resistance is a common phenomenon, occurring in approximately 30% of the general population (Ioannou et al. 2007, Haffner and Miettinen 1997).

During early pregnancy, glucose tolerance is normal or even slightly improved and peripheral insulin sensitivity is normal compared with the non-pregnant state. Women in early pregnancy are more sensitive to the blood glucose-lowering effect of exogenously administered insulin than they are later on in the second and third trimesters (Catalano et al. 1991, 1992, 1993). The cause of enhanced insulin secretion in early pregnancy is uncertain.

In normal pregnancy, maintenance of normoglycaemia, despite insulin resistance, is achieved by way of enhanced insulin secretion by the pancreatic β-cells (Catalano et al. 1991). A likely contributing factor is hyperplasia of pancreatic islets (Van Assche et al. 1978).

The physiological meaning and ultimate causes of pregnancy-induced insulin resistance are not known. However, pregnancy-induced factors, together with pro-inflammatory cytokines, like TNFα, are thought to play a role in development of insulin resistance (Radaelli et al. 2003). In addition, significant crosstalk exists between insulin and the SNS: muscle sympathetic activity is increased by insulin and acute activation of the SNS decreases insulin sensitivity (Rowe et al. 1981, Berne et al. 1992, Avogaro et al. 1996).

Coagulation

Normal pregnancy is associated with changes in all aspects of haemostasis, including increases in the concentrations of most clotting factors (e.g. factors I, V, VII, VIII, IX, X, XII) and adhesion protein vWF, decreasing concentrations of some of the natural anticoagulants (like protein S), and diminishing fibrinolytic activity (increased fibrinogen). In addition, platelets are activated during normal pregnancy. These changes lead to hypercoagulation, although anticoagulatory alterations also occur, such as increases in thrombomodulin and TFPI (Hellgren 2003, Brenner 2004).

The most marked changes are seen around term and in the immediate post-partum period, and haemostasis is normalized approximately 4 weeks after delivery (Franchini 2006). Pregnancy-induced haemostatic changes help in meeting the haemostatic challenge associated with delivery. At the same time they increase the risk of thromboembolism. This risk may be exacerbated by the concomitant presence of certain conditions, such as immobilisation or maternal thrombophilia.

Inflammation

Pregnancy represents a physiological state of activation of the innate immune system characterized by elevated circulating concentrations of many acute-phase inflammatory markers (von Versen-Hoeynck et al. 2009, see also Table 2). Leucocytosis is present from the early stages of pregnancy and it increases towards the third trimester, and there is evidence for leukocyte activation (Belo et al. 2005, Smarson et al. 1996). Cytokines secreted from both immune and non-immune (e.g. decidual, chorionic) cells are thought to be involved (and even essential) in several aspects of
reproduction, including implantation, growth and development of trophoblasts, protection of the foetal allograft from maternal rejection, foetal fuel availability and parturition (Bowen et al. 2002, Saito 2000, Barbour et al. 2007).

The placenta plays a marked role in synthesis and release of inflammatory proteins and virtually all known cytokines have been demonstrated to be expressed in the placenta (Bowen et al. 2002). In pregnant women, there is a shift from cell-mediated to humoral immunity; cytokines associated with humoral immunity and produced by Th2 cells (IL-4, IL-5, IL-6) predominate over those associated with cell-mediated immunity and produced by Th1 cells (IL-2, interferon γ, TNFα) (Challis et al. 2009).

Obesity, particularly abdominal obesity, during pregnancy is associated with low-grade tissue inflammation, glucose intolerance and insulin resistance, with the result that pregnancy complications, such as gestational diabetes, are commoner in obese than non-obese women (Ramsay et al. 2002, Martin et al. 2009). Defects in the insulin signalling cascade have been described in pregnant obese women (Colomiere et al. 2009), but the biology of adipose tissue, and its links to insulin resistance, circulating inflammatory cytokines, and adverse pregnancy outcome need more investigation.

Sympathetic nervous system

Normal pregnancy is associated with increased SNS activity, especially in the 3rd trimester. This is demonstrated by assessment of circulating NA, HRV and muscle sympathetic activity recordings (Kaaja and Pöyhönen-Alho 2006). In the first trimester, vagal modulation overcomes sympathetic modulation, and the balance is shifted towards higher sympathetic and lower vagal modulation in the third trimester. This biphasic behaviour of the ANS during normal pregnancy may be affected by the balance between the haemodynamic changes and aorto-caval compression caused by the enlarging gravid uterus (Kuo et al. 2000).
Gestational diabetes

Definition

Gestational diabetes (GDM) is defined as glucose intolerance of variable severity with onset or first recognition during pregnancy (Metzger et al. 2007).

Pathophysiology

Like all forms of hyperglycaemia, GDM is characterized by insufficient insulin production to meet insulin demands. Pancreatic β-cell dysfunction is pivotal but the causes are not fully defined. According to today’s knowledge, the underlying defects seem to be chronic rather than of acute onset: during pregnancy physiological insulin resistance unmasks an already existing β-cell defect which can still be detected years after pregnancy, even when glucose tolerance seems normal (Seghieri et al. 2007).

Insulin resistance

Two forms of insulin resistance exist in women who develop GDM: baseline insulin resistance presenting before pregnancy, and ‘superimposed’ physiological insulin resistance of late pregnancy. In GDM, the former is exacerbated by the latter. Even though women with GDM, as a group, are slightly more insulin-resistant than normal pregnant women (Metzger et al. 2007), they are far from homogeneous as regards the insulin resistance trait. The severity of insulin resistance in GDM varies widely between patients and is independent of degree of metabolic control (Natali et al. 2006). These findings explain the great variability of results among studies concerning insulin resistance in GDM.

Pancreatic β-cell function

Women with GDM have lower insulin secretion for their degree of insulin resistance when compared with normal pregnant women (Di Gianni et al. 2003). A large body of evidence indicates that most (but not all) women with GDM have abnormal pancreatic β-cell function, especially as regards first-phase insulin secretion (Grill and Efendric 1987, Catalano et al. 1991, Miyakoshi et al. 2010). The severity of β-cell dysfunction seems to correlate with the severity of glucose intolerance in women with GDM (Miyakoshi et al. 2010). Women with normal first-phase β-cell
responsiveness usually present with milder forms of GDM and are at relatively low risk of developing diabetes after pregnancy (Buchanan et al. 1990). A small minority of women with GDM appear to have β-cell dysfunction of autoimmune origin.

**Genetic factors**

Even though GDM clusters in families and common variants in several candidate genes have been demonstrated to increase the risk of GDM (Shaat and Groop 2007), the contribution of genetics is not well established. Increased prevalence of T2D in first-degree relatives of patients with GDM as well as a major role of ethnicity in the likelihood of developing GDM during pregnancy underline genetic predisposition (Martin et al. 1985, Anna et al. 2008).

**Role of the placenta and inflammation**

The placenta is a rich source of multiple substances, including steroids, peptides and lipid-derived molecules. During GDM, the placenta suffers remarkable structural and functional alterations. GDM has been reported to elicit major changes in the expression profile of placental genes for insulin signalling, lipid and glucose metabolism as well as inflammatory responses (Radaelli et al. 2003, Colomiere et al. 2009, Lappas et al. 2005, Lappas et al. 2010). GDM is characterized by pronounced systemic inflammation compared with normal pregnancy (Bowen et al. 2002, Barbour et al. 2007, Challis et al. 2009, Volpe et al. 2007), and TNFα mRNA in skeletal muscle has been shown to be elevated in women with GDM even though circulating inflammation profiles have been similar to those in women with normoglycaemic pregnancies (Kim et al. 2008, Thomann et al. 2008, Friedman et al. 2008). It has been hypothesized that low-grade tissue inflammation and insulin resistance, associated with excessive adiposity and pancreatic β-cell dysfunction, could favour the development of GDM, leading to type 2 diabetes and cardiovascular diseases later in life (Volpe et al. 2007).

**Modifiable factors**

Modifiable factors associated with the risk of GDM include physical activity and overweight/obesity. Although the effect of physical activity on prevention of T2D is clear, only a few studies have been conducted concerning GDM. However, these studies have demonstrated that physical activity before and during pregnancy is associated with a decreased risk of developing GDM (Tobias et al. 2011). Pre-pregnancy weight and GDM are closely linked, in that overweight and obese women have higher risks of developing GDM compared with women of normal weight.
(Torloni et al. 2009). It has been calculated that a reduction in BMI of 1 kg/m² reduces the risk of GDM by 1% (Torloni et al. 2009). Moreover, inverse correlation between socioeconomic status and GDM has been identified across various ethnic groups (Anna et al. 2008).

Diagnosis and screening

Both diagnosis and screening of GDM vary greatly worldwide. Recently, the International Association of Diabetes and Pregnancy Study Groups has formulated guidelines for screening and diagnosis of diabetes in pregnancy. Key components of the recommendations include screening of high-risk women at the first encounter for pre-gestational diabetes, universal screening at 24–28 weeks' gestation, and use of the 75-g oral glucose tolerance test (Leary et al. 2010). Other widely used screening methods are risk-based screening and the 50-g glucose challenge test followed by the 100-g oral glucose tolerance test. In Finland, national guidelines for screening and diagnosis of GDM were recently introduced (Finnish Current Care Guidelines, 2008) and are schematically presented in Figure 5.

Epidemiology

The incidence of GDM varies among populations with recent prevalence estimates ranging from less than 2% in Sweden, to 22% in Sardinia (Hunt and Schuller 2007). In Finland, GDM was diagnosed in 2006 in 8.5% and in 2009 in 9.2% of parturients (STAKES birth register). Recent reports from the U.S., Canada, Australia and Europe provide evidence for an increasing prevalence of GDM (Cheung and Byth 2003, ACOG Practice Bulletin 2004, Dabelea et al. 2005, Hunt and Schuller 2007, Anna et al. 2009, Davenport et al. 2010). Even though these reports suggest an epidemic of GDM, estimates of global trends in incidence are made difficult by wide variability of glucose tolerance testing (glucose load, number and timing of tests, glycaemic thresholds). The increasing prevalence of obesity, particularly in youth, has been proposed as one of the causal factors for the apparent increase in GDM. Another noticeable factor is the survival of female infants whose birth weights are at the extremes of normal weights. The altered insulin action and insulin secretory capacity of these individuals may predispose them to development of GDM later in life.
Figure 5. Screening and diagnosis of gestational diabetes in Finland, Current Care Guideline. (www.kaypahoito.fi)

- **<25 years**
  - nulliparous
  - BMI 18.5–25
  - no 1st degree family history of T2D

- **<40 years**
  - multiparous
  - no previous GDM
  - no previous infant > 4.5 kg
  - BMI <25

- **Other pregnant women**

- **Suspicion of GDM e.g. glucosuria**

- **2 h 75 g OGTT at the time of suspicion**

- **NORMAL**

- **2-h 75-g OGTT at 24–28 weeks**

- **NORMAL**

- **Plasma glucose (mmol/l)**
  - fasting ≥ 5.3
  - 1h ≥ 10.0
  - 2h ≥ 8.6

- **NO GDM**

- **GDM**
Long-term health concerns

Type 2 diabetes
Over the long term, insulin secretion may deteriorate in relation to chronic insulin resistance present in women with GDM, eventually leading to T2D. Two large meta-analyses on the development of T2D following GDM report a risk ratio of 7.43 and prevalence of up to 70% (Bellamy et al. 2009, Kim et al. 2002). Progression increases steeply within the first five years, reaching a plateau soon thereafter (Kim et al. 2002). Once diagnosed with GDM, women appear to progress to T2D mostly regardless of ethnicity (Kim et al. 2002, Bellamy et al. 2009). Elevated fasting glucose levels observed during pregnancy strongly predict T2D; women with the highest glucose levels seem to have the highest risk (Kim et al. 2002). Other risk factors include high maternal age, high pre-pregnancy BMI, early diagnosis of GDM and need for insulin therapy (Kim et al. 2002, Ogonowski and Miazgowski 2009).

Cardiovascular diseases
Individuals with T2D have a more than 20% risk of developing a new major coronary event within 10 years following diagnosis (Haffner et al. 1998). In addition to T2D, women with pregnancy complicated by GDM more often develop hypertension, unfavourable lipid profiles and metabolic syndrome compared with those with a history of normoglycaemic pregnancy (Bentley-Lewis 2009). Surrogate measures of CVD, such as impaired endothelial function, cardiac autonomic dysfunction and elevated levels of CRP, have been shown to be associated with GDM compared with women with a normal pregnancy history (Bentley-Lewis 2009).

Health consequences of GDM in the second generation
Long-term health concerns affect not only women with GDM but also their offspring. Maternal glucose, but not insulin, crosses the placenta easily, resulting in intrauterine hyperglycaemia and compensatory foetal insulin secretion. This modification of the endocrine environment of the foetus has been suggested to change the programming of foetal metabolism, also affecting later childhood, and adulthood (Pedersen 1977, Radaelli et al. 2009, Luo et al. 2010). The risks of an overweight condition, metabolic syndrome, pre-diabetes and T2D are markedly increased in the offspring of women with GDM when compared with the background population, even in low-risk populations (Dabelea et al. 2000, Clausen et al. 2009, Vääräsmäki et al. 2009, Damm 2009).
Prevention

Although GDM is a well-established risk factor of T2D, many women with GDM are either unaware or do not believe that they specifically are at risk of developing the disease (Bentley-Lewis et al. 2008). This might affect compliance with risk-reduction intervention. In order to increase awareness and acceptance of the personal risk of the development of T2D, public education campaigns as well as other easily available information are needed. Lifestyle modification studies have shown promising results in preventing or diminishing progression to T2D in several populations (Knowler et al. 2002, Tuomilehto et al. 2001, Pan et al. 1997), but no studies have specifically been conducted to assess healthy lifestyle intervention in women with GDM. In a recent meta-analysis, pre-pregnancy and early pregnancy physical activity were found to be protective against development of GDM, with odds ratios of 0.45 and 0.76, respectively (Tobias et al. 2011).

Concerning prevention of T2D in women of fertile age, the benefit of breast-feeding is seldom considered, even though it seems to be protective, with a twofold reduction in the development of diabetes among both women with a history of GDM (Kjos et al. 1993) as well as women in the general population (Taylor et al. 2005).

Pharmacological prevention of T2D after GDM is not currently recommended, but results from randomized placebo-controlled studies on glitazones (Buchanan et al. 2002, Xiang et al. 2006) as well as metformin (Knowler et al. 2002) in women with a history of GDM have shown marked risk reduction in progression to T2D. The incidence of diabetes has been shown to be reduced by 30% with metformin and by more than 50% with glitazones.

Sympathetic nervous system, insulin sensitivity and inflammation after menopause

The occurrence of CAD is different in women and men, as onset begins approximately 10 years later in women (Mosca et al. 2007). A major increase in the incidence of CVD in women coincides with menopause, suggesting a role of oestrogen deficiency in this change. Studies on oestrogen therapy have also supported beneficial effects of hormonal therapy on insulin action and glucose tolerance (Szmuilowicz et al. 2009), but this hypothesis still remains to be proven and the possible underlying mechanisms investigated.
Sympathetic nervous system

Hormonal changes along a woman’s life span are associated with complex fluctuations in cardiovascular regulatory mechanisms, including the SNS. The first hormonal ‘storm’, puberty, is associated with variations in catecholamines (Weise et al. 2002). From menarche onwards, SNS activity correlates with plasma oestrogen levels, sympathetic control being higher during the luteal phase (high hormonal state) compared with the early follicular phase of a menstrual cycle (Hirshoren et al. 2002). Ageing has been shown to increase muscle sympathetic activity (Fagius and Wallin 1993) and the increase is more marked in women than in men, especially during menopause (Narkiewicz et al. 2005, Hart et al. 2009). Because the incidence of CVD in women increases markedly after the menopause, getting closer to that of men, these findings have led to the hypothesis that the SNS may play a role in the development of CVD in women after menopause. This view has also been supported by some work in humans suggesting that oestrogen decreases sympathetically mediated responses (Sudhir et al. 1997, Vongpatanasin et al. 2001, Kaaja and Pöyhönen-Alho 2006). However, these results are not conclusive (Niskanen et al. 2002). Thus, ageing of the homeostatic mechanisms involved in cardiovascular regulation, including the SNS, may be independent of sex, and sex steroid effects of endogenous and exogenous oestrogen may be mediated by different mechanisms (Narkiewicz et al. 2005, Lavi et al. 2007).

Insulin resistance

According to the current literature it is not unequivocal whether or not menopause is associated with increased insulin resistance. In a mouse model, gradual ovarian failure is associated with increasing insulin resistance (Romero-Aleshire et al. 2009), but studies in humans show conflicting results (Kaaja and Pöyhönen-Alho 2006, Lejskova et al. 2010, An et al. 2009). It is estimated that middle-aged women gain approximately 0.5 kg/year owing to an increase in abdominal obesity (Crawford et al. 2000, Toth et al. 2000) and at the same time muscle mass is reduced (Poehlman et al. 1993). In addition, postmenopausal years are associated with reduced energy expenditure (Poehlman et al. 1995). Menopause per se may thus not strictly be related to a decrease in insulin sensitivity, but be associated with unfavourable changes in body composition, especially an increase in central body fat (Denino et al. 2001, Manco et al. 2006, Aasen et al. 2009).
Inflammation

It is well established that menopause coincides with changes in levels of systemic markers of inflammation as well as the incidence and progression of specific inflammatory conditions, such as coronary heart disease, asthma and inflammatory bowel disease (Gameiro et al. 2010, Gilliver 2010). These changes can be partly explained by an increase in intra-abdominal fat after menopause (Lee et al. 2009), but abrupt cessation of ovarian oestrogen biosynthesis per se seems to play a role. Oestrogen is mainly considered to be an antioxidant, anti-inflammatory agent, via mechanisms that are not fully elucidated. At the cellular level, oestrogen influences chemotaxis, activation and secretion of inflammatory cytokines and adhesive interactions between inflammatory cells and the endothelium (Gilliver 2010). However, clinical studies have failed to show conclusive anti-inflammatory effects of oestrogen (Gilliver 2010, Gameiro et al. 2010). On the contrary, long-term oral (but not transdermal) use of hormonal therapy has been shown to increase plasma CRP levels (Shifren et al. 2008, Lowe et al. 2001, Zegura et al. 2003 and 2006).

Postmenopausal hypertension

Menopause is accompanied by an increase in BP independent of age, BMI and other confounding factors, including oestrogen therapy (Staessen et al. 1989, Zanchetti et al. 2005). Hypertension (followed by left ventricular hypertrophy) is the main risk factor contributing to the increase in cardiovascular morbidity and mortality in postmenopausal women, with a prevalence of around 60% in women older than 65 years (Taddei 2009). The high prevalence of hypertension in older women is largely due to the progressive stiffening of the arterial structure which accompanies the ageing process in both sexes. However, the abrupt fall in circulating oestrogen concentrations might independently contribute to the rise in BP through a direct effect on the arterial wall and activation of the renin-angiotensin system (Vongpatanasin et al. 2001, Yanes et al. 2010).
AIMS OF THE STUDY

Because the pathophysiology of GDM and mechanisms behind the transition of GDM to T2D are incompletely understood, we aimed to study

1. Nocturnal SNS activity in pregnancy complicated by GDM compared with normal pregnancy
2. Subclinical inflammatory cytokines, and associations of the SNS with these cytokines
3. Coagulation, and associations of coagulation and fibrinolysis markers with the SNS
4. Effect of central inhibition of the SNS on inflammatory cytokines

SUBJECTS AND METHODS

Subjects

The subjects in the two studies in this thesis are presented in Table 5.

Methods

Publications I–III

Treatment of GDM followed the clinical guidelines of Helsinki University Central Hospital. Dietary treatment, consisting of 1600–1800 kcal/day, was started immediately after the diagnosis of GDM. If fasting or 1-h postprandial plasma glucose levels exceeded 5.5 or 7.8 mmol/l, respectively, insulin treatment was initiated. Initially, NPH insulin in a single evening dose was used and, in cases of postprandial hyperglycaemia, short-acting insulin prior to meals was introduced.
Table 4. Study subjects.

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of subjects</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDM</td>
<td>41</td>
<td>at least one 75-g 2-h OGTT value abnormal: ≥5.3 mmol/l (fasting state), ≥10.0 mmol/l at 1 h and ≥8.6 mmol/l at 2 h</td>
<td>smoking, clinical signs of infection, medication affecting glucose metabolism, the sympathetic nervous system</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCs</td>
<td>22</td>
<td>normal OGTT, normal BP, BMI &gt;25kg/m²</td>
<td></td>
</tr>
<tr>
<td>NPCs</td>
<td>14</td>
<td>no history of pathological blood glucose values or hypertension, BMI &gt;25kg/m²</td>
<td>coagulation</td>
</tr>
</tbody>
</table>

**Publications I–III**

- **GDM**
  - normal BP
  - hypertensio****
    - No. of subjects: 41
    - Inclusion criteria: at least one 75-g 2-h OGTT value abnormal: ≥5.3 mmol/l (fasting state), ≥10.0 mmol/l at 1 h and ≥8.6 mmol/l at 2 h
    - Exclusion criteria: smoking, clinical signs of infection, medication affecting glucose metabolism, the sympathetic nervous system

- **PCs**
  - No. of subjects: 22
  - Inclusion criteria: normal OGTT, normal BP, BMI >25kg/m²
  - Exclusion criteria: 

- **NPCs**
  - No. of subjects: 14
  - Inclusion criteria: no history of pathological blood glucose values or hypertension, BMI >25kg/m²
  - Exclusion criteria: coagulation

**Publication IV**

- **Moxonidine**
  - No. of subjects: 56
  - Inclusion criteria: natural menopause
  - Exclusion criteria: use of any other medication

- **Atenolol**
  - No. of subjects: 56
  - Inclusion criteria: hypertension (mean of 3 sitting diastolic BP measurements 95–114 mmHg), BMI >25kg/m²
  - Exclusion criteria: use of any other medication

* previous diagnosis of hypertension, or diastolic BP values ≥90 mmHg and/or systolic values ≥140 mmHg during the first trimester of pregnancy.

** cessation of menstruation 1–5 years earlier, serum follicle-stimulating hormone ≥30 IU/l and a menopause sign score ≥1 (each menopausal symptom – hot flashes, palpitations, insomnia, irritability and depression – graded as 0=absent, 1= mild, 2= moderate, 3= severe).

All study subjects spent one night in hospital and the same investigator (M.P-A.) performed all the clinical studies. To standardize menstrual cycle-dependent SNS activity, the NPCs were studied at mid-cycle, which was confirmed by way of menstrual history and vaginal ultrasonographic examination. For blood sampling a cannula was inserted into a cubical vein. Blood samples were drawn during the night at 24, 4 and 7 h to determine plasma NA, AM and insulin concentrations.

The coagulation variables examined included prothrombin time (PT), thrombin time (TT), activated partial thrombin time (APTT), fibrinogen D-dimer, FVIII activity (FVIII:C), vWF antigen
(vWF:Ag), ristocetin cofactor activity (vWF:RCo) and collagen binding activity (vWF:CB) as well as primary haemostasis (assessed via PFA-100®).

The inflammatory cytokines studied were CRP, IL-6, insulin growth factor-1 (IGF-1), SAA, sex hormone-binding globulin (SHBG), alpha-1 glycoprotein (AGP) and cortisol. At the same time a fingertip blood sample was taken for blood glucose determination. The study participants were asked to sleep or to stay in bed in a lying position during the whole study period and to list all obligatory standing-up times. Supine blood pressure was measured twice, 5 minutes apart, in the morning before getting up. All study subjects underwent 2-channel overnight ECG recording for assessment of HRV. The HRV analyses were initiated at 23 h and continued until 8 h. We chose this night-time recording period rather than a full 24 hours in order to standardize, as far as possible, the study environment as recommended for clinical studies (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996).

The study was approved by The Ethics Committee of Gynaecology and Obstetrics of the hospital district of Helsinki and Uusimaa, and written informed consent was obtained from all participants.

**Blood sample analyses**

Blood was collected into specific tubes (NA and AM: cooled EDTA tubes; assays for coagulation parameters: cooled 109 mM (3.2%) trisodium citrate; assays for inflammation: cooled serum tubes), centrifuged immediately at 4 °C for 15 minutes at 3000 g and the separated plasma was frozen in aliquots at -80 °C until assayed. NA and AM samples were transported to New Zealand on dry ice and assays were carried out at Christchurch Hospital, New Zealand, in duplicate, and all samples from each individual were measured in a single assay (Mefford et al. 1981, Lewis et al. 1998). Coagulation and inflammation analyses were performed using commercially available kits at Biomedicum laboratory, Helsinki and the laboratory of Helsinki University Hospital (HUSLAB) according to the manufacturer’s instructions. The methods for blood sample analyses and the reference ranges are listed in Table 6.

**HRV analyses**

ECG tapes were initially visually analyzed to label the QRS complexes and classified with respect to normal or ventricular extrasystoles. Analysis of HRV was carried out with custom-made software
Table 6. Methods for blood sample analyses, and reference ranges.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Method</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated partial thromboplastin time</td>
<td>Actin® FSL reagent (Siemens Healthcare Diagnostics, Germany)</td>
<td>23–33 s</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>ELISA (B-Bridge International, USA)</td>
<td>not available</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>Radioimmunoassay (Lewis et al. 1998)</td>
<td>2.7–10.1 pmol/l</td>
</tr>
<tr>
<td>Alpha -1 glycoprotein</td>
<td>Immunoturbidimetric (Tina-quant®, Roche Diagnostics)</td>
<td>500–1200 mg/l</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Immunochemiluminometric method (Abbott Diagnostics, UK)</td>
<td>150–650 nmol/l</td>
</tr>
<tr>
<td>D-dimer</td>
<td>Immunoturbidimetric assay (Tina-quant®, Roche Diagnostics)</td>
<td>&lt;0.5 mg/l</td>
</tr>
<tr>
<td>Factor VIII activity</td>
<td>Pathromtin® SL and FVIII deficient plasma (Siemens Healthcare Diagnostics, Germany)</td>
<td>52–148 %</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Modification of the Clauss method with Multifibre® U reagent (Siemens Healthcare Diagnostics, Germany)</td>
<td>1.7–4.0 g/l</td>
</tr>
<tr>
<td>Insulin</td>
<td>Immunofluorometry (Toivonen et al. 1986)</td>
<td>2.3–26 mU/l</td>
</tr>
<tr>
<td>Insulin growth factor-1</td>
<td>ELISA (R&amp;D Systems)</td>
<td>40–174 ng/ml</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>ELISA (R&amp;D Systems)</td>
<td>0.5–10.0 pg/ml</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>Reverse phase HPLC with electrochemical detection (Mefford et al. 1981)</td>
<td>470–3800 pmol/l</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>Nycotest® PT reagent (Medinor, Norway)</td>
<td>70–130%</td>
</tr>
<tr>
<td>Sensitive C-reactive protein</td>
<td>Immunoturbidimetry (Tina-quant®, Roche Diagnostics)</td>
<td>0.05–3 mg/ml</td>
</tr>
<tr>
<td>publication II</td>
<td>Immunonefleometry (CardioPhase hsCRP reagent, BN ProSpec, Siemens Healthcare Diagnostics, Germany)</td>
<td>&lt;1 mg/l low risk</td>
</tr>
<tr>
<td>publication IV</td>
<td></td>
<td>1–3 mg/l intermediate risk</td>
</tr>
<tr>
<td>Serum amyloid A</td>
<td>ELISA (Biosource International, USA)</td>
<td>&gt;3 mg/l high risk</td>
</tr>
<tr>
<td>Sex hormone-binding globulin</td>
<td>ELISA (IBL International, Germany)</td>
<td>61–599 ng/ml</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>BC Thrombin Reagent (Siemens Healthcare Diagnostics, Germany)</td>
<td>17–25 s</td>
</tr>
<tr>
<td>Tumour necrosis factor α</td>
<td>ELISA (R&amp;D Systems, UK)</td>
<td>1.2–15.3 pg/ml</td>
</tr>
<tr>
<td>Tumour necrosis factor α receptor II</td>
<td>ELISA (R&amp;D Systems, UK)</td>
<td>not available</td>
</tr>
<tr>
<td>von Willebrand factor antigen</td>
<td>vWF Ag latex reagent (Siemens Healthcare Diagnostics, Germany)</td>
<td>51–169 %</td>
</tr>
<tr>
<td>von Willebrand factor collagen-binding activity</td>
<td>ELISA (Technoclone GmbH, Austria)</td>
<td>50–170 %</td>
</tr>
<tr>
<td>von Willebrand factor ristocetin cofactor</td>
<td>BC® von Willebrand Reagent (Siemens Healthcare Diagnostics, Germany)</td>
<td>44–183 %</td>
</tr>
</tbody>
</table>
suitable for moving-time HRV analysis (Aalto University, Helsinki, Finland, heikki.vaananen@tkk.fi). The following two time-domain components of HRV were measured:

SDNN, representing the standard deviation of the nightly sinus node cycle length, and the standard deviation of the average normal-to-normal intervals for each 5-min period, SDANN. Frequency-domain analysis was performed using fast Fourier transformation. Before this the normal-to-normal time series was re-sampled with a boxcar window, convoluted with a Hanning window, and the linear trend was removed. The frequency-domain indices VLF, LF and HF as well as the LF to HF ratio were then calculated. In addition to absolute values, LF and HF data were transformed into normalized units (nu). Frequency domain indices were analyzed over the whole 9-h period and additionally over a one-hour period before each blood sampling.

Publication IV

This multicentre (10 centres) and multinational (Finland, Sweden, Lithuania) study design was a prospective and double-blind comparison of moxonidine and atenolol. After a 4-week placebo run-in phase the patients were randomly assigned for 8 weeks to 0.3 mg moxonidine twice or 50 mg atenolol once daily. Matching placebo tablets were used in the atenolol group to achieve twice-daily administration. During the course of the study, including the 4-week run-in period, the patients were not to receive any other forms of antihypertensive medication, which were discontinued for 2 weeks, and in the case of β-blockers, for 1 month prior to the run-in period. Compliance to the medication was regarded as sufficient if the subject took at least 85% of the scheduled medication. Blood samples for analysis of the inflammatory markers (CRP, TNFα, TNFα receptor II, IL-6 and adiponectin) were taken at the beginning of the study (after the run-in period) and after 8 weeks of medical treatment.

All samples were analyzed in the same laboratory (Yhtyneet Laboratoriot, Helsinki, Finland). If the result of an inflammation marker test was lower or higher than the sensitivity of the test used, the result was expressed as the lowest or highest sensitivity detection value, correspondingly. The lowest detection rate used for TNFα was less than 0.06 pg/ml, and the highest rate for IL-6 more than 10 pg/ml and for adiponectin more than 25 000 pg/ml. Participants with CRP levels of > 10 mg/l at baseline and/or after the follow-up period were excluded because of the risk of infection as a confounding factor.
Statistics

Publications I–III

A sample size of 19 patients per group, in detecting a difference in plasma concentrations of NA of 300 pmol/l (SD 320) between the groups, was calculated by using a two-sided $t$-test with $\alpha=0.05$ and power=80%. The variables were initially analyzed by using repeated measurements analysis of variance (RMANOVA) for difference over time, group and group and time interactions. Variables measured only at baseline were analyzed by means of a simple analysis of variance (ANOVA) model. Paired or unpaired Mann–Whitney $U$-tests were used to compare groups and different time points as appropriate. Concerning CRP and SAA measurements, logarithmic transformations were carried out because of reasonably wide variability of single measurements of these variables and to achieve more reliable information on comparisons between the groups. Pearson’s (publication I) and Spearman's correlation coefficients (publications II and III) were calculated from the entire data. Linear regression analysis was performed to evaluate the effect of BMI on the measured variables. Analyses were carried out using SAS® software, version 8.2, SAS Institute Inc., Cary, NC, USA (publication I) and PASW® software, version 18.0 for Windows; SPSS Inc., Chicago, IL, USA (publications II and III).

Publication IV

Sample size estimation and power calculation were performed with a power of 80% to reject the null-hypothesis at a 5% level. The standard deviation of the target parameter, diastolic BP, is known from other studies to be 6.5 mmHg. Assuming a clinically relevant difference to be 4 mmHg, and using a one-sided $t$-test, a sample size of 57 patients per treatment group was required. The difference between treatment groups in distribution of change in inflammatory markers was analyzed using nonparametric analysis of covariance with baseline values as covariates. The study was accepted by the Ethics Committees of each participating centre.
RESULTS

Characteristics of the study groups

Publications I–III

Four subjects were excluded from the analyses because of occasional smoking not apparent at the time of recruitment (2 women in the GDM group with hypertension and 1 with normal BP, and 1 woman in the PC group). Baseline characteristics of the study groups are presented in Table 6. The women in the GDM group appeared to be more obese when compared with PCs and NPCs but gained less weight during pregnancy, most probably as a result of dietary treatment. The NPCs had had fewer previous pregnancies than the other groups. Eight of the 38 GDM subjects (21%) needed insulin in addition to dietary treatment. No differences were found in baseline characteristics between diet-only and insulin-treated GDM subjects, with the exception of higher levels of blood glucose in insulin-treated subjects at all time points (24 h, 5.7 vs. 4.9 mmol/l, \( p=0.0004 \); 4 h, 5.7 vs. 4.9 mmol/l, \( p=0.0007 \) and 7 h, 5.7 vs. 4.8 mmol/l, \( p<0.0001 \)).

Five of the hypertensive GDM subjects used oral labetalol hydrochloride (combined \( \alpha \)- and \( \beta \)-blocking agent) as antihypertensive medication. Because of the potential effect of this medication on SNS activity these patients were excluded from analyses of SNS (HRV, NA and their associations). For technical reasons (faulty Holter recorder), reliable HRV data were unavailable in 27 subjects, leaving reliable HRV data from 47 women, 26 in the GDM group (18 with normal BP and 8 with hypertension), 12 in the PC group and 9 in the NPC group.

Publication IV

Nineteen women were excluded because of lack of data (time of menopause, diastolic BP, BMI at baseline) or a blood sample. Four participants in the atenolol and two in the moxonidine group were excluded because one or both CRP values exceeded 10 mg/l. Thus the final study population included 44 patients with atenolol and 43 with moxonidine treatment. The study groups were comparable according to their baseline characteristics (Table 7).
Table 7. Characteristics of the study groups, mean (SD)

<table>
<thead>
<tr>
<th>Publications I–III</th>
<th>GDM (n=41)</th>
<th>PCs (n=22)</th>
<th>NPCs (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.0 (5.6)</td>
<td>29.5 (4.9)</td>
<td>30.4 (6.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 (6.0)</td>
<td>26.9 (3.0)</td>
<td>26.5 (6.3)</td>
</tr>
<tr>
<td>Parity</td>
<td>2.2 (1.4)</td>
<td>1.2 (0.9)</td>
<td>0.5 (0.4)</td>
</tr>
<tr>
<td>Weight gain during pregnancy (kg)</td>
<td>11.0 (6.3)</td>
<td>16.3 (8.9)</td>
<td></td>
</tr>
<tr>
<td>Duration of pregnancy at time of study (d)</td>
<td>211.8 (30.6)</td>
<td>216.0 (16.9)</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>119.7 (17.7)</td>
<td>113.3 (10.8)</td>
<td>113.4 (8.2)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71.5 (10.7)</td>
<td>67.1 (7.1)</td>
<td>69.9 (9.5)</td>
</tr>
<tr>
<td>Blood glucose at 24 h (mmol/l)</td>
<td>5.1 (0.6)</td>
<td>5.1 (0.6)</td>
<td>6.1 (0.7)</td>
</tr>
<tr>
<td>Blood glucose at 4 h</td>
<td>5.1 (0.6)</td>
<td>4.9 (0.4)</td>
<td>5.4 (0.5)</td>
</tr>
<tr>
<td>Blood glucose at 7 h</td>
<td>5.0 (0.5)</td>
<td>4.6 (0.3)</td>
<td>5.3 (0.6)</td>
</tr>
<tr>
<td>Number of upright positions</td>
<td>2.0 (1.1)</td>
<td>1.5 (1.4)</td>
<td>1.2 (1.2)</td>
</tr>
<tr>
<td>Breathing frequency/min</td>
<td>29 (6)</td>
<td>26 (8)</td>
<td>25 (5)</td>
</tr>
</tbody>
</table>

\(^a p<0.05 \) GDM vs. PCs and NPCs
\(^b p<0.001 \) NPCs vs. GDM and PCs
\(^c p<0.05 \) PCs vs. GDM and NPCs

GDM, gestational diabetes; PCs, pregnant controls; NPCs, non-pregnant controls; BMI, body mass index; BP, blood pressure

<table>
<thead>
<tr>
<th>Publication IV</th>
<th>ATENOLOL (n=43)</th>
<th>MOXONIDINE (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.4 (2.8)</td>
<td>53.4 (3.1)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.9 (4.0)</td>
<td>29.3 (4.0)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>101 (5)</td>
<td>102 (5)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>160 (16)</td>
<td>160 (18)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.5 (0.8)</td>
<td>5.5 (0.9)</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>11.9 (6.3)</td>
<td>11.6 (7.0)</td>
</tr>
<tr>
<td>Insulin sensitivity (Matsuda index)</td>
<td>4.2 (2.1)</td>
<td>4.7 (2.8)</td>
</tr>
</tbody>
</table>
Sympathetic nervous system

Noradrenaline

Nocturnal changes of NA concentrations are shown in Figure 6. In both the GDM group and the PCs, mean NA levels increased significantly during the early morning hours from 4 to 7 h, with no difference between the groups. The change from 24 to 7 h was different in the GDM and NPC groups (p<0.001), and in the PC and NPC groups (p=0.008). The change from 4 h to 7 h was significantly different between PCs and NPCs (p=0.022) and nearly significant between women with GDM and the NPCs (p=0.067).

In a sub-analysis, no differences in NA levels were found within the GDM group between women with normal BP vs. hypertension.

Figure 6. Time-associated nocturnal changes of noradrenaline concentrations in the study groups. Mean±SD.

* GDM: change from 24 to 7, p=0.001 and change from 4 to 7, p<0.01
** PCs: change from 4 to 7, p<0.01
*** NPCs: change from 24 to 7, p<0.05 and change from 24 to 4, p<0.05
Heart rate variability

Time- and frequency-domain analyses of HRV are presented in Figure 7. Both pregnant groups showed lower SDNN and SDANN values as well as lower LF values than the NPCs. The GDM group exhibited lower HF values than the NPCs. No differences existed in LF/HF between the groups. Although the GDM and PC groups did not differ in respect of 9-hour HRV, a difference in the change profiles of HFnu and LFnu was evident (Figure 8). HFnu and LFnu values as well as their ratios remained unchanged in the GDM vs. the PC and NPC groups.

Figure 7. Time- and frequency-domain indices of HRV

* indicates significance. GDM vs. NPCs: p < 0.001

*p < 0.001
Inflammatory markers

Conclusive results regarding inflammatory parameters are presented in Table 1 of the original publication II. CRP concentrations were higher during pregnancy compared with the non-pregnant state. Also, concentrations of IGF-1, SHBG and cortisol (except at 24 h) were higher in pregnant than in non-pregnant subjects. No differences were seen in levels of any of the studied variables between the GDM and PC groups. However, concentrations of CRP and SAA remained almost constant during the night in the GDM group, whereas variation was seen in normal pregnancy (Figure 8).

The only correlation between NA and inflammatory cytokines was seen with SAA in the GDM group ($r=0.43$, $p=0.016$). No such correlation existed in PCs and NPCs.
Changes in inflammatory markers in the study in which we compared 8-week use of atenolol or moxonidine in hypertensive overweight postmenopausal women are presented in Table 8. Levels of CRP and IL-6 did not differ between the groups. Concentrations of TNFα increased in the atenolol group but decreased in the moxonidine group and the difference was even greater as regards adiponectin, the concentrations of which decreased dramatically in the atenolol group but did not change in moxonidine treatment group. In regression analysis, only treatment group independently affected changes in levels of adiponectin and TNFα.

Table 8. Changes in inflammatory parameters from baseline to 8 weeks in subjects using atenolol and moxonidine. Mean (SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ATENOLOL n=44</th>
<th>MOXONIDINE n=43</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>0.7 (3.0)</td>
<td>0.5 (6.3)</td>
<td>ns</td>
</tr>
<tr>
<td>TNFα (ng/l)</td>
<td>0.05 (0.1)</td>
<td>-0.03 (0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNFα-RII (ng/l)</td>
<td>4.4 (233.6)</td>
<td>52.6 (370.6)</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6 (ng/l)</td>
<td>0.19 (0.7)</td>
<td>0.03 (0.6)</td>
<td>ns</td>
</tr>
<tr>
<td>adiponectin (μg/l)</td>
<td>-1733.3 (2754.7)</td>
<td>13.7 (1921.9)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The effects of atenolol compared with those of moxonidine on BP and insulin sensitivity in the same study population have been published earlier (Kaaja et al. 2007). Mean BP decreased significantly during the 8-week period in both atenolol- (9.5 mmHg, p<0.001) and moxonidine-treated patients (6.2 mmHg, p<0.001). In patients in whom blood pressure was reduced, insulin sensitivity improved in the moxonidine group (p=0.02), but not in the atenolol group.
**Insulin**

Nocturnal alterations in insulin levels are shown in Figure 9. Concentrations of insulin remained almost constant in the GDM group between the sampling times, whereas distinct variation (decrease towards morning) was evident in the PCs and NPCs. Insulin concentrations were higher in the GDM group than in the NPCs at 4 am and 7 am, whereas no statistical difference existed between PCs and NPCs.

Figure 9. Nocturnal variation of insulin concentrations. Mean±SD.

* * p<0.01 GDM vs. NPCs
**Adrenomedullin**

Plasma concentrations of ADM were higher in pregnant than in non-pregnant women at all time points ($p<0.001$), as shown in Figure 10. No difference existed between the GDM group and PCs.

Figure 10. Nocturnal variation of adrenomedullin concentrations. Mean±SD.

* $p<0.001$ GDM and PCs vs. NPCs
Haemostatic variables

Coagulation and fibrinolysis

Pregnancy was associated with changes in all aspects of haemostasis towards enhanced coagulation activity compared with the non-pregnant state (see publication III, Table 2). No differences existed in coagulation and fibrinolysis variables or in clotting times in the GDM and normal pregnancy groups, with the exception of FVIII:C, which was lower in the GDM group than in the PCs. The differences were statistically significant at 4 and 7 h, and approached significance at 24 h ($p=0.07$). In addition, the levels of all variables of vWF appeared to be lower in the GDM group than in the PCs, even though the differences were not statistically significant (Table 9).

Thrombin time and APTT increased slightly in the GDM group from 4 to 7 h, but not in the other groups. A moderate correlation was found between NA concentrations and APTT ($r=0.28$, $p=0.02$).

BMI had a modest effect on fibrinogen levels ($r=0.12$–0.13, $p=0.006$–0.009; linear regression), but no or only a minor effect on the other variables.

Platelet function

In platelet function studies, no differences existed between GDM and normal pregnancy. In pregnant vs. non-pregnant subjects, a shorter time in clot formation at midnight, i.e. increased platelet activity, was found, as measured by PFA-100®. In non-pregnant women, the time needed for clot formation shortened markedly towards the morning, whereas no change was seen in pregnant women (Figure 11). In linear regression analysis, BMI explained only 1–5% of the clot formation times.
Table 9. Nocturnal variation of Factor VIII, von Willebrand Factor, thrombin time and activated partial thromboplastin time. Mean (SD).

<table>
<thead>
<tr>
<th>Sampling time (at)</th>
<th>GDM (n=38)</th>
<th>PCs (n=21)</th>
<th>NPCs (n=14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GDM vs. PCs</td>
<td>GDM vs. NPCs</td>
<td>PCs vs. NPCs</td>
</tr>
<tr>
<td>FVIII:C (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>126.2 (29.8)</td>
<td>140.7 (25.8)</td>
<td>78.6 (26.2)</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>119.9 (24.3)</td>
<td>143.9 (42.7)</td>
<td>78.4 (30.5)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>7</td>
<td>119.5 (32.0)</td>
<td>140.0 (24.3)</td>
<td>75.9 (25.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>vWF:Ag (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>179.0 (75.9)</td>
<td>193.5 (57.6)</td>
<td>83.1 (29.0)</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>174.7 (73.6)</td>
<td>189.7 (61.1)</td>
<td>75.5 (25.2)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>7</td>
<td>179.3 (72.0)</td>
<td>189.2 (58.6)</td>
<td>75.4 (21.4)</td>
<td>ns</td>
</tr>
<tr>
<td>vWF:RCo (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>183.8 (68.2)</td>
<td>197.4 (74.8)</td>
<td>85.6 (35.1)</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>179.6 (67.4)</td>
<td>197.6 (81.1)</td>
<td>80.6 (35.7)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>7</td>
<td>186.4 (66.3)</td>
<td>199.2 (78.5)</td>
<td>83.9 (32.2)</td>
<td>ns</td>
</tr>
<tr>
<td>vWF:CB (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>160.3 (76.8)</td>
<td>171.2 (64.2)</td>
<td>69.1 (17.4)</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>148.9 (66.6)</td>
<td>165.8 (62.6)</td>
<td>72.7 (25.4)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>7</td>
<td>148.4 (63.3)</td>
<td>160.8 (65.3)</td>
<td>68.2 (22.4)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Thrombin Time (s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15.5 (1.7)</td>
<td>15.0 (0.7)</td>
<td>17.8 (1.8)</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>15.5 (1.2)</td>
<td>15.1 (0.6)</td>
<td>18.1 (1.5)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>7</td>
<td>17.6 (13.2)</td>
<td>15.1 (0.8)</td>
<td>17.7 (1.5)</td>
<td>ns</td>
</tr>
<tr>
<td>APTT (s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25.2 (2.2)</td>
<td>25.0 (1.4)</td>
<td>27.1 (1.9)</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>25.3 (1.5)</td>
<td>24.3 (1.8)</td>
<td>27.4 (2.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>7</td>
<td>26.1 (5.4)</td>
<td>24.6 (1.5)</td>
<td>27.5 (2.1)</td>
<td>ns</td>
</tr>
</tbody>
</table>

GDM, gestational diabetes; PCs, pregnant controls; NPCs, non-pregnant controls; FVIII:C, factor VIII activity; vWF, von Willebrand factor, vWF:Ag, vWF antigen, vWF:RCo, vWF ristocetin cofactor activity; vWF:CB, vWF collagen-binding activity; ns, non-significant
Correlation analyses

We found no statistically significant correlations between NA concentrations and coagulation variables. As expected, FVIII and vWF correlated strongly in all groups at all sampling times ($r=0.66–0.78$, $p<0.001$). Concentrations of AM correlated strongly with vWF:Ag ($\rho=0.83$, $p=0.000$), vWF:RCo ($\rho=0.80$, $p=0.001$) and vWF:CB ($\rho=0.68$, $p=0.010$) as well as with FVIII:C ($\rho=0.82$, $p=0.003$) in NPCs. However, these associations did not exist in either of the pregnant groups.
Sub-analysis of women with GDM with and without hypertension

In a sub-analysis, we compared, within the GDM group, women with essential hypertension (n=18, 44%) with those having normal BP (n=23). The differences observed are shown in Table 10. No differences existed in measurements of sympathetic activity (NA, HRV) or in levels of AM between the groups. Insulin concentrations were higher in subjects with hypertension at midnight but not thereafter. Concerning subclinical inflammatory markers, SHBG concentrations were lower and AGP levels higher in hypertensive subjects, but statistical significance between the groups was reached only at 24 h. In the coagulation analyses, fibrinogen levels were elevated in hypertensive women at all time points and APTT was shorter at 4 h.

Table 10. Observed differences between subjects with gestational diabetes with and without hypertension. Mean (SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>GDM with hypertension</th>
<th>GDM with normal BP</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>18.2 (8.1)</td>
<td>12.5 (8.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 h</td>
<td>13.8 (3.6)</td>
<td>12.5 (7.2)</td>
<td>0.49</td>
</tr>
<tr>
<td>7 h</td>
<td>13.4 (5.7)</td>
<td>11.7 (5.9)</td>
<td>0.39</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>197.6 (67.0)</td>
<td>238.8 (34.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 h</td>
<td>200.4 (46.7)</td>
<td>226.1 (33.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>7 h</td>
<td>210.1 (46.0)</td>
<td>234.6 (28.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>AGP (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>593.6 (74.9)</td>
<td>525.1 (114.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 h</td>
<td>571.2 (70.3)</td>
<td>520.2 (116.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>7 h</td>
<td>587.1 (78.6)</td>
<td>526.5 (125.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td></td>
<td></td>
<td></td>
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<td>24 h</td>
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DISCUSSION

Sympathetic nervous system in gestational diabetes

Autonomic nervous system dysfunction (mostly interpreted as overactivation of the SNS) has been considered to be one of the main contributors to the development of CVD (Mueller et al. 2007, McNicholas et al. 2007), and this study was established in order to investigate SNS activity in GDM. We found no difference in NA concentrations in GDM compared with normal pregnancy although the NA temporal profiles were somewhat different. Both pregnant groups showed comparable increases in NA concentrations during the early morning hours, whereas no such increase was seen in non-pregnant healthy women. In HRV analysis, comparably reduced total variability was seen in both pregnant groups in comparison with the non-pregnant state. These results indicate, firstly, an early morning increase in overall sympathetic activation and withdrawal of parasympathetic modulation of the heart during pregnancy, and, secondly, the applicability of HRV analysis as a mirror of overall SNS activity during pregnancy.

An increase in NA concentrations towards morning in normal pregnancy has been demonstrated earlier (Kaaja et al. 1999). Concerning differences in SNS activity in GDM compared with normal pregnancy, only a few studies can be found. Sánches-Margalet et al. found increased concentrations of NA in a single measurement in a fasting blood sample after supine rest for 30 minutes in 12 women with GDM, who were compared with 11 women with normoglycaemic pregnancy (Sanchez-Margalet et al. 1998). Concentrations of NA in their work were approximately 3 times higher than ours in GDM and 5-fold in subjects with normal pregnancy. In addition to differences between NA assessment methods used, this difference could be explained by greater activity of the SNS in women studied daytime, but possibly also (at least to some degree) by physical activity and other stresses during the day.

Weissman et al. studied 12 women with GDM and 15 pregnant women with normal OGTT results, before and after a 100-g OGTT, and found in 10-minute ECG recordings a higher LF/HF ratio in the GDM group at baseline (Weissman et al. 2006). Ten-minute ECG recordings were also used in 51 women with GDM and 28 with normal pregnancies in a study by Heiskanen et al. They found higher LFnu and LF/HF in the 3rd trimester than postpartum in all subjects, with no difference between GDM and normal pregnancy. They concluded that GDM does not result in cardiovascular dysfunction when it is in good balance (Heiskanen et al. 2010). In contrast, Gasic et al.
demonstrated reduced HRV in women with a history of GDM one year after pregnancy during both daytime and at night (Gasics et al. 2007).

Our results are in concordance with those of Heiskanen et al. in that GDM is not associated with reduced HRV compared with normal pregnancy. However, we did not find any differences in frequency domain values. Although reduced time domain indices in HRV are generally accepted as measures of SNS activation (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996), interpretation of the frequency domain indices needs some caution. No general agreement exists on physiological bases of all frequency domain indices. HF power is agreed to represent parasympathetic activity, but the nature of LF is more controversial. LF is considered by some (Malliani et al. 1991, Kamath and Fallen 1993, Montano et al. 1994) as a marker of sympathetic modulation (especially when expressed in normalized units) and by others (Akselrod et al. 1985) as a parameter that includes both sympathetic and parasympathetic influences. Based on the latter view, the LF/HF ratio is considered to mirror sympathovagal balance or to reflect sympathetic modulation. This rationale has been challenged on the basis of studies which have demonstrated that parasympathetic blockade markedly reduces the LF component of HRV (Taylor et al. 1998). In our study, no differences were seen in LF/HF between any of our study groups or any time-points within the groups, although total variability (SDNN, SDANN) showed significant parasympathetic withdrawal in the pregnant groups. Possible explanations could be contemporaneous parasympathetic and sympathetic withdrawal in pregnant groups and/or increased parasympathetic and sympathetic activation in NPCs. Regardless of the physiological interpretation of HF, LF and LF/HF, our finding of loss of natural fluctuation in these indices in GDM is of interest.

Increased circulating NA levels can modulate cardiac autonomic regulation in healthy subjects (Tulppo et al. 2005) and somewhat different NA temporal profiles between the GDM and PC groups could explain, at least partly, the difference in cardiac ANS modulation. A more likely factor behind this difference, however, is pre-diabetic status of women with GDM.

Our finding could indicate early disturbance of autonomic neural control in GDM. Reduced variation could precede apparent changes in HRV seen in diabetic patients (Vinik et al. 2007). Indeed, this hypothesis is supported by a study concerning postprandial thermogenesis (PPT) and NA responses to a meal in women with a history of GDM. The SNS is thought to play an important role in regulating PPT (Astrup et al. 1989). In a study by Kousta et al., no difference existed in the
amount of PPT in women with previous GDM compared with women with normal pregnancy, although the shape of the PPT curve revealed consistent delay in PPT, insulin, and NA responses to a meal in the study group (Kousta et al. 2002). The authors interpreted this as a metabolic defect preceding the reductions in absolute indices in further advanced disease.

Our study was performed in well-standardized resting circumstances, minimizing external factors potentially disturbing SNS assessment. In addition, SNS activity was studied by using two different methods, which gave synergistic results. The heavy study protocol, which required an overnight stay in hospital for both the subject as well as the researcher, limited the number of subjects. This was especially seen in healthy non-pregnant women, of whom we were able to recruit only 14. Hence the results concerning pregnant vs. non-pregnant women may be considered to be preliminary. Unfortunately, we lost one third of the Holter recordings because of technical problems, which leaves the possibility that some additional differences could have been seen with a greater number of such recordings.

The most effective means of prevention of CVD include lifestyle modification, such as healthy diet and regular physical exercise. This may be partly explained via reduction of SNS activity (Bisquolo et al. 2005, Amano et al. 2001, O’Dea et al. 1982). While waiting for further evidence on the efficacy of diet and exercise on the incidence of GDM and long-term health of these women, a healthy lifestyle should be encouraged in all women with GDM.

**Inflammation**

Inflammation represents a physiological process maintaining tissue homeostasis and comprising complex crosstalk between different biological systems making use of messengers to hasten (positive feedback) and attenuate (negative feedback) the process. In healthy individuals, this chain of events generates intra-individual variation/fluctuation of various participating hormones and proteins. In early stages of certain diseases, this sensitive feedback system may be disturbed long before clinical signs and symptoms of the disease appear. Interest concerning the role of low-grade tissue inflammation in CVD has been focused mainly on long-term changes in levels of inflammatory markers: CRP has been found a strong independent predictor of adverse cardiac events and death both in patients with heart disease as well as in apparently healthy men and
women (Ridker et al. 1997). Short-term variation in the concentrations of inflammatory markers has not received attention in this respect.

As expected, we found increased low-grade tissue inflammation in pregnant vs. non-pregnant women. No differences were found in the concentrations of inflammatory cytokines in GDM vs. normal pregnancies. However, nocturnal variation of CRP was significantly smaller in cases of GDM than in normal pregnancy. Concentrations CRP remain relatively stable over long periods of time (Ridker et al. 2007) and they lack systematic diurnal variation in healthy individuals (Meier-Ewert et al. 2001). The magnitude of variation observed among women in our study was comparable to that reported previously (Meier-Ewert et al. 2001).

In general, the sources of short-term variation of biological agents are assumed to be external to the workings of the organism – temperature, food ingestion, physical activity etc. Advanced analysis of some serial data on naturally varying agents has suggested that the source of biological variation is endogenous rather than exogenous and that it encompasses deterministic behaviour (Kroll et al. 1999). Our finding of reduced nocturnal variability of CRP, and SAA, suggests disturbance in regulation of inflammatory homeostasis in GDM and this may provide new information regarding the role of inflammatory markers in the underlying pathogenesis. Reduced (nocturnal) variability could precede sustained increases of these inflammatory factors and indicate a need for immediate action against overt low-grade tissue inflammation and its possible deleterious consequences. In the light of the results on attenuated heart ANS modulation presented above, this hypothesis (and the need for preventive actions) becomes more substantial.

Moxonidine, an imidazoline receptor agonist, decreases blood pressure by inhibiting central nervous sympathetic activity (Ernsberger et al. 1997, Wenzel et al. 1998). Through this mechanism, moxonidine has been suggested to improve insulin sensitivity in hypertensive overweight postmenopausal women (Kaaja et al. 2007). We investigated, in that study, the effect of moxonidine on low-grade tissue inflammation. Ideally, as regards this thesis, such a study would have been performed in our pregnant women with and without GDM. However, the use of moxonidine is contraindicated during pregnancy. Similarly as younger women with GDM, postmenopausal overweight hypertensive women are at an increased risk of CVD. Thus there is a rationale for applying the results in both groups.
We found favourable changes in the inflammatory profile in women who had used moxonidine for 8 weeks compared with those taking the β-blocking agent atenolol. In the moxonidine group, concentrations of TNFα were reduced and those of adiponectin remained unchanged, whereas in the atenolol group levels of TNFα increased and those of adiponectin decreased dramatically. These findings indicate, primarily, that centrally acting sympatholytic agents may be beneficial in treatment of postmenopausal women with hypertension and clustering of multiple risk factors for CVD. Secondly, they strengthen the earlier view that SNS and inflammation are tightly connected.

While all medication calls for careful consideration during pregnancy, a healthy lifestyle is of importance for the mother or the child. SNS activity can be reduced by regular physical exercise and diet and, according to our results, reduction in SNS activity might affect low-grade tissue inflammation. These changes, in turn, could affect the pathophysiology of GDM. Indeed, the risk of GDM has been shown to be inversely proportional to the degree of physical activity before and during pregnancy (Tobias et al. 2011). Thus regular daily exercise should be recommended for all pregnant women (Hegaard et al. 2007).

**Coagulation**

Our results confirmed the well-known fact that pregnancy is a state of hypercoagulation. In a large epidemiological study, women diagnosed with GDM were at 4-fold higher risk for VTE during pregnancy when compared to women with normal glucose tolerance (Jacobsen et al. 2008). The mechanisms behind this observation are unknown. Only a few studies have examined coagulation in women with GDM, and the results have been somewhat controversial. Women with GDM have been found to exhibit elevated PAI-1 levels compared with women with normal pregnancies (Akinzi et al. 2008), whereas other investigators have not found such a difference (Winzer et al. 2004, Bellart et al. 1998). One study revealed higher levels of tissue plasminogen activator, D-dimer and thrombin-antithrombin complexes as well as lower levels of proteins C and S, suggesting increased coagulation and decreased anticoagulation activity in GDM (Bellart et al. 1998). In a recent study of 150 women with GDM and 100 normoglycaemic controls, Abdel et al. found higher fibrinogen as well as protein S levels in cases of GDM (Abdel et al. 2010).

We found no differences in the concentrations of the coagulation parameters studied between the GDM and the normal pregnancy group, with the exception of lower FVIII:C in the GDM group.
FVIII participates in coagulation via the contact activation pathway, together with coagulation factors I, II, V, IX, XI and XII. APTT mirrors the synergy of these factors. We found a small early morning increase in APTT, suggesting prolongation of endothelial-associated coagulation and slightly increased TT, suggesting weakened fibrin formation in cases of GDM compared with normal pregnancy. In addition, slightly lower vWF levels in subjects with GDM were seen. Together, these findings indicate attenuation of the contact activation pathway in GDM – an observation not supporting a role of a contact activation pathway behind increased VTE risk in GDM (Jacobsen et al. 2008). Of interest is the positive correlation between NA and APTT found in this study, suggesting an association of the SNS with the contact activation pathway of coagulation.

To our knowledge, there is only one published study assessing FVIII in pregnancies complicated by GDM. Scholtes and co-workers found an increased FVIII antigen/activity ratio in GDM, but only after 32 weeks of pregnancy (Scholtes et al. 1983). As the duration of pregnancy in our subjects was shorter, the results are not quite comparable. The lower FVIII:C values observed in this study may be due to somewhat lower vWF levels, since vWF is the carrier of FVIII. One possibility as regards the decreased amounts of these endothelial coagulation factors could be increased consumption during pregnancy, especially in GDM, but this presumption remains to be investigated. As vWF is considered to reflect impaired endothelial function, our results speak against endothelial impairment in GDM, at least in our study population. Considering the strong association between AM and vWF in non-pregnant subjects, discussed below, dissociation of the AM-vWF axis during pregnancy could suggest a role of AM in decreased levels of FVIII and vWF in pregnancy vs. the non-pregnant state.

Adrenomedullin is expressed by endothelial cells, the intensity of expression being dependent on the degree of haemodynamic shear stress. It is thus suggested to have a role in local modulation of vascular tone and regulation of coagulation (Marutsuka 2003). This role seems to be antithrombotic, based on previous findings demonstrating the inhibitory action of AM on expression of TF and PAI-1 (Sugano et al. 2001) and an increasing effect on tissue factor pathway inhibitor (Sugano et al. 2001, Liu et al. 2007). Adrenomedullin levels are elevated in normal pregnancy (Wilson et al. 2004), which was also demonstrated in this study. No difference in AM levels was seen between normal and GDM pregnancies. This is in concordance with results published by Di Iorio et al. (Di Iorio et al. 2001). We found a remarkably strong positive correlation between AM and both quantitative and qualitative vWF markers in non-pregnant
healthy women, a finding compatible with an earlier view that AM is a relevant regulatory factor in human coagulation. However, these correlations were lost during pregnancy. The dissociation of the AM-vWF axis during pregnancy may be added to earlier findings of aberrant regulation of coagulation during gestation. The precise mechanisms and significance behind this finding, however, remain to be elucidated.

Elevated circulating levels of catecholamines increase platelet activity both in vitro and in vivo (Clayton et al. 1963, O’Brien 1963, Anfossi and Trovati 1996, Mustonen et al. 1996, Westerbacka et al. 2002). Even though platelets were more activated at midnight in our pregnant subjects, platelet activity seemed to attenuate, not increase, with increasing NA levels towards the morning. In addition, we found no correlation between NA and platelet function. A possible explanation for these contradictory findings could be higher insulin concentrations during pregnancy. Insulin is known to interfere with NA action in the central nervous system (Robertson et al. 2010). In addition, it attenuates crucial steps in thrombus formation (Westerbacka et al. 2002). Finally, insulin has been shown to increase PFA-100 clotting time (Westerbacka et al. 2002). In the present study, pregnant women had higher insulin concentrations than the NPCs. Insulin could, therefore, through platelets, serve as a beneficial protective factor against thrombus formation during pregnancy.

**Gestational diabetes with and without hypertension**

GDM and hypertensive disorders of pregnancy (pregnancy-induced hypertension, pre-eclampsia) are associated, one increasing the occurrence of the other (Landon et al. 2009, Benley-Lewis 2009). Hypertension is associated with insulin resistance and hyperinsulinaemia (Whaley-Connel and Sowers 2009), changes in levels of inflammatory cytokines (Liu et al. 1996, Chae et al. 2001, Peeters et al. 2001) and changes in markers of coagulation and fibrinolysis (Lee 1997, Poli et al. 2000). These findings support the clinical association between hypertension and metabolic disorders and increased risk of thromboembolic complications (Gress et al. 2000, Felmeden and Lip 2005). Our results are in concordance with these findings; we found higher levels of insulin and increased insulin resistance (lower concentrations of SHBG) as well as higher concentrations of AGP in women with GDM combined with hypertension. However, these differences seemed to disappear towards the morning. One possible explanation for this phenomenon could be physiological diurnal changes during the
early morning hours (e.g. increased cortisol secretion, activation of the SNS) which may interfere and/or mask the underlying differences.

SHBG has been demonstrated to be a strong independent risk factor of the development of T2D in women (Lindstedt et al. 1991). Recent genetic studies indicate that SHBG and sex hormones are also involved in the aetiology of type 2 diabetes (Ding et al. 2006, Perry et al. 2010). Thus, lower serum SHBG concentrations in GDM, combined with hypertension, could indicate an increased risk of T2D in these women. Earlier studies on SHBG in GDM as well as in hypertensive pregnancy complications have, however, shown controversial results (Thomann et al. 2008, Spencer et al. 2005, Kopp et al. 2001, Mc Eldruff et al. 2005, Yu et al. 2004, Wolf et al. 2002).

The presence of elevated concentrations of fibrinogen in hypertensive women with GDM in this study is in concordance with earlier findings in hypertensive non-pregnant subjects (Lee 1997). The absence of other differences in the studied coagulation parameters could be explained by young age and relatively mild hypertension in our study subjects, since the degree of a prothrombotic state has been shown to correlate with the degree and duration of hypertension (Lip and Blann 2000). Another explanation may be the small number of subjects in the sub-study.
CONCLUSIONS

The occurrence of GDM during pregnancy is an important risk factor for later development of T2D and CVD. Understanding the pathophysiology of GDM could give us tools for primary prevention, because the delay in the development of T2D and CVD after GDM may be decades. The present study was aimed at investigation of the possible role of the SNS in GDM and the association of the SNS with insulin, AM, low-grade tissue inflammation and coagulation.

The results indicated

1. Similar overall SNS activity but disturbed heart SNS modulation (as evidenced by reduced rhythmicity of HRV) in GDM compared with normal pregnancy.

2. Comparable low-grade tissue inflammation in GDM and normal pregnancy without uniform associations between NA and the studied cytokines. Decreased nocturnal variation of CRP concentrations in GDM vs. normal pregnancy.

3. Lower FVIII:C in GDM than in normal pregnancy; no significant correlations between NA and coagulation variables.


Our results thus did not support our a priori hypothesis of increased SNS activity, low-grade tissue inflammation and enhanced coagulation in GDM. Nor did they support straight associations between the SNS, inflammation and coagulation. Measurement of a large number of different variables revealed an interesting finding showing reduced nocturnal variation in HRV frequency domain indices, CRP, SAA and insulin in GDM. A new hypothesis was set: GDM is associated with early disturbance of autonomic and humoral control that may precede apparent changes with advancing disease, i.e. transition of GDM to T2D (Figure 12). If this hypothesis, in the future, proves valid, further understanding of the regulatory mechanisms could provide new strategies for
prevention of metabolic diseases after GDM. Our results support incorporation of the SNS as one of the targets in this battle.
Figure 12. Hypothesis of reduced neuronal and humoral variability in gestational diabetes preceding type 2 diabetes with increases of markers of inflammation and autonomic dysregulation.

Time axis for transition from healthy pregnancy to type 2 diabetes
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Maritta Pöyhönen-Alho
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