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**INSULIN SENSITIVITY IN PRE-ECLAMPSIA:  
RELATIONSHIPS TO LEPTIN, HOMOCYSTEINE  
AND ACTIVIN-INHIBIN**

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**Academic Dissertation**

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Helsinki 1999

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ISBN 952-45-8698-0 (PDF version)  
Helsingin yliopiston verkkojulkaisut 1999

***To Jouko, Martti and Jaakko***

# CONTENTS

<b>LIST OF ORIGINAL PUBLICATIONS .....</b>	<b>6</b>
<b>ABBREVIATIONS .....</b>	<b>7</b>
<b>I INTRODUCTION .....</b>	<b>8</b>
<b>II REVIEW OF THE LITERATURE .....</b>	<b>9</b>
1. PRE-ECLAMPSIA .....	9
1.1 <i>Definitions</i> .....	9
1.2 <i>Pathogenesis and risk factors</i> .....	10
1.2.1 Placental factors and endothelial dysfunction .....	10
1.2.2 Maternal factors .....	12
1.2.3 Familial tendency .....	13
2. INSULIN RESISTANCE AND INSULIN RESISTANCE SYNDROME .....	13
2.1 <i>Measurement of insulin sensitivity</i> .....	15
2.2 <i>Implications of insulin resistance</i> .....	16
3. CARBOHYDRATE METABOLISM IN PREGNANCY .....	18
3.1 <i>Normal pregnancy</i> .....	18
3.2 <i>Pre-eclampsia</i> .....	18
4. LIPIDS AND LIPOPROTEINS IN PREGNANCY .....	19
4.1 <i>Normal pregnancy</i> .....	19
4.2 <i>Pre-eclampsia</i> .....	20
5. LEPTIN .....	21
5.1 <i>Leptin in a non-pregnant state</i> .....	21
5.2 <i>Leptin in pregnancy</i> .....	21
6. HOMOCYSTEINE .....	22
6.1 <i>Homocysteine in a non-pregnant state</i> .....	22
6.2 <i>Homocysteine in pregnancy</i> .....	22
7. ACTIVIN A AND INHIBIN A IN PREGNANCY .....	23
8. THE METABOLIC EFFECTS OF ANTIHYPERTENSIVE DRUGS .....	23
9. IMPACT OF A HISTORY OF PRE-ECLAMPSIA ON PREGNANCY OUTCOME AND CARDIOVASCULAR MORBIDITY .....	24
9.1 <i>Subsequent pregnancies</i> .....	24
9.2 <i>Cardiovascular morbidity</i> .....	25
<b>III AIMS OF THE STUDY .....</b>	<b>27</b>
<b>IV SUBJECTS AND METHODS .....</b>	<b>28</b>
1. SUBJECTS .....	28
3. STATISTICAL ANALYSIS .....	31
<b>V RESULTS .....</b>	<b>32</b>
1. INSULIN SENSITIVITY, LIPIDS AND LIPOPROTEINS DURING AND AFTER PRE-ECLAMPTIC PREGNANCY (I) .....	32
2. SERUM LEPTIN DURING AND AFTER PRE-ECLAMPTIC PREGNANCY. RELATIONSHIP TO INSULIN SENSITIVITY (II) .....	36
3. PLASMA HOMOCYSTEINE DURING AND AFTER PRE-ECLAMPTIC PREGNANCY. RELATIONSHIP TO INSULIN SENSITIVITY (III) .....	39

4. SERUM ACTIVIN A, INHIBIN A AND PRO- $\alpha$ C INHIBIN DURING PRE-ECLAMPTIC PREGNANCY. RELATIONSHIP TO INSULIN SENSITIVITY (IV) .....	40
5. INSULIN SENSITIVITY, LIPIDS AND LIPOPROTEINS DURING ISRADIPINE AND METOPROLOL TREATMENT IN PRE-ECLAMPTIC PREGNANCY (V).....	43
6. GLUCOSE TOLERANCE, LIPIDS AND LIPOPROTEINS IN WOMEN WITH PRIOR PRE-ECLAMPTIC PREGNANCY (VI).....	43
7. SEX HORMONES AND ENDOTHELIAL MARKERS IN WOMEN WITH PRIOR PRE-ECLAMPTIC PREGNANCY (VII) .....	45
<b>VI DISCUSSION .....</b>	<b>45</b>
<b>VII SUMMARY AND CONCLUSIONS .....</b>	<b>52</b>
<b>ACKNOWLEDGEMENTS .....</b>	<b>54</b>
<b>REFERENCES.....</b>	<b>56</b>

## LIST OF ORIGINAL PUBLICATIONS

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

- I Kaaja R, Laivuori H, Laakso M, Tikkanen MJ, Ylikorkala O. Evidence of a state of increased insulin resistance in preeclampsia. *Metabolism*, 1999; 48: 892-896.
- II Laivuori H, Kaaja R, Koistinen H, Andersson S, Karonen S-L, Koivisto V, Ylikorkala O. Leptin during and after preeclamptic or normal pregnancy: its relation to insulin sensitivity. *Metabolism*, in press.
- III Laivuori H, Kaaja R, Turpeinen U, Viinikka L, Ylikorkala O. Plasma homocysteine levels elevated and inversely related to insulin sensitivity in preeclampsia. *Obstetrics and Gynecology* 1999; 93: 489-493.
- IV Laivuori H, Kaaja R, Turpeinen U, Stenman U-H, Ylikorkala O. Serum activin A and inhibin A in pre-eclampsia: no relation to insulin sensitivity. *Br J Obstet Gynaecol*, in press.
- V Laivuori H, Laakso M, Tikkanen MJ, Cacciatore B, Ylikorkala O, Kaaja R. Short-term metabolic effects of isradipine and metoprolol in preeclampsia. *J Hypertens* 1999; 17: 1189-1194.
- VI Laivuori H, Tikkanen MJ, Ylikorkala O. Hyperinsulinemia 17 years after preeclamptic first pregnancy. *J Clin Endocrinol Metab* 1996; 81: 2908-2911.
- VII Laivuori H, Kaaja R, Rutanen E-M, Viinikka L, Ylikorkala O. Evidence of high circulating testosterone in women with prior preeclampsia. *J Clin Endocrinol Metab* 1998; 83: 344-347.

## ABBREVIATIONS

BMI	body mass index
CVD	cardiovascular disease
ET-1	endothelin 1
FFA	free fatty acid
GDM	gestational diabetes mellitus
GLUT	glucose transporter protein
HDL	high-density lipoprotein
HK	hexokinase
HPL	human placental lactogen
IL	interleukin
LDL	low-density lipoprotein
LPL	lipoprotein lipase
NIDDM	non-insulin-dependent diabetes mellitus
OGTT	oral glucose tolerance test
PCOS	polycystic ovary syndrome
PGI <sub>2</sub>	prostacyclin
TNF	tumor necrosis factor
TXA <sub>2</sub>	thromboxane A <sub>2</sub>
VLDL	very low-density lipoprotein

# I INTRODUCTION

Pre-eclampsia is a pregnancy-specific multisystem disorder characterised by reduced placental perfusion, vascular endothelial dysfunction, and activation of the coagulation cascade (Roberts and Redman 1993). Hypertension and proteinuria are the clinical symptoms of pre-eclampsia which are easiest to recognise. Pre-eclampsia is predominantly a disease of primigravidae, characterised also by typical renal lesions, which disappear after delivery. Occurring in approximately 3% of pregnancies in the western world (Saftlas *et al.* 1990), it is one of the leading causes of maternal death (Grimes 1994).

The insulin resistance syndrome, a cluster of metabolic changes including insulin resistance, glucose intolerance, hyperinsulinaemia, hypertriglyceridaemia, decreased high-density lipoprotein (HDL) cholesterol, and hypertension, is a powerful risk factor as regards cardiovascular disease (CVD) (Reaven 1988). Most of these metabolic changes are also observed in pre-eclampsia (Kaaja *et al.* 1995, Hubel *et al.* 1996, Lorentzen *et al.* 1998). Another factor possibly affecting endothelial function, an elevated plasma homocysteine concentration, appears to indicate an increased risk of CVD in later life in men and non-pregnant women (Graham *et al.* 1997). It is also known that endocrine disorders, such as polycystic ovary syndrome (PCOS) and non-insulin-dependent diabetes mellitus (NIDDM) are associated with insulin resistance (Davidson 1995). Both of these diseases are also associated with an increased risk of pre-eclampsia (Berkowitz 1998). All this may imply that there could be a connection between insulin resistance and pre-eclampsia.



## II REVIEW OF THE LITERATURE

### 1. Pre-eclampsia

#### 1.1 Definitions

The American College of Obstetricians and Gynecologists (1972) recommended classification of hypertension during pregnancy into chronic hypertension, pre-eclampsia, pre-eclampsia superimposed on chronic hypertension, and transient hypertension. Later, two international committees used slightly different definitions for pre-eclampsia (Table 1). In addition to elevated blood pressure and proteinuria, impaired liver function, increased serum uric acid, decreased platelet count, and symptoms and signs, such as headache, visual disturbances, epigastric pain, and pulmonary oedema are considered particularly ominous (Gifford *et al.* 1990). Most investigators consider pre-eclampsia and transient hypertension to be distinct syndromes (Chesley 1980). Although the latter may also be a pre-proteinuric phase of pre-eclampsia, it can be a recurrence of chronic hypertension which abated in mid-pregnancy, or a manifestation of latent essential hypertension (Gifford *et al.* 1990).

**Table 1. The definition of pre-eclampsia according to two international committees**  
**The International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray 1988)**

<b>Pre-eclampsia</b>	Hypertension and proteinuria developing during pregnancy, labour, or puerperium in a previously normotensive nonproteinuric woman
<i>Hypertension</i>	A. Diastolic blood pressure of $\geq 110$ mm Hg on any one occasion, or B. Diastolic blood pressure of $\geq 90$ mm Hg on two occasions $\geq 4$ hours apart
<i>Proteinuria</i>	The diastolic blood pressure is taken as the point of muffling (phase IV) of the Korotkoff sounds Total protein $\geq 0.3$ g in one 24-hour urine collection Two "clean-catch-midstream" or catheter specimens of urine collected $\geq 4$ hours apart with 1. 1 g albumin per litre or $\geq 2+$ on reagent strip 2. 0.3 g albumin per litre or 1+ on reagent strip in a sample of specific gravity $< 1.03$ and pH $< 8$

**National High Blood Pressure Education Program Working Group (Gifford *et al.* 1990)**

<b>Pre-eclampsia</b>	Increased blood pressure accompanied by proteinuria, edema, or both
<i>Hypertension</i>	$\geq 140/90$ mm Hg after 20 weeks' gestation Systolic blood pressure increases $\geq 30$ mm Hg, or diastolic blood pressure increases $\geq 15$ mm Hg
<i>Proteinuria</i>	$\geq 0.3$ g in a 24-hour specimen $\geq 1+$ on dipstick
<i>Edema</i>	Clinically evident swelling Rapid increase in weight

## 1.2 Pathogenesis and risk factors

Pre-eclampsia has been called “a disease of theories“ which well depicts the fact that the aetiology of pre-eclampsia has remained poorly understood (Table 2). Yet growing evidence indicates genetic or immunological conflicts between the mother and the fetus as aetiological factors of this disease (Dekker and Sibai 1998). Pre-eclampsia is increased in association with oocyte donation, when the foetus genetically differs from the mother (Söderström-Anttila *et al.* 1998), and when a man has already fathered a pre-eclamptic pregnancy (Lie *et al.* 1998). In some studies barrier contraceptive use (Klonoff-Cohen *et al.* 1989) and donor insemination (Smith *et al.* 1997) are also predisposing factors as regards pre-eclampsia, although contradicting data also exists (Mills *et al.* 1991, Laivuori *et al.* 1998). It is equally clear that the placenta and maternal constitutional factors contribute to the aetiology of this disease (Roberts 1998).

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**Table 2. Some modern hypotheses of the aetiology of pre-eclampsia**

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Immunological maladaptation	(Redman 1991)
Genetic disease	(Cooper <i>et al.</i> 1993)
Placental ischaemia, increased trophoblast deportation	(Smarason <i>et al.</i> 1993)
Very-low-density lipoprotein-toxicity	(Arbogast <i>et al.</i> 1994)
Distinct placental and maternal genesis, endothelial injury is the point of convergence	(Ness and Roberts 1996)
Excessive maternal inflammatory response to pregnancy	(Redman <i>et al.</i> 1999)

---

### 1.2.1 Placental factors and endothelial dysfunction

Pre-eclampsia can develop without a foetus as is the case in hydatidiform mole (Gifford *et al.* 1990). A theory regarding the placenta as a primary origin of pre-eclampsia stems from evidence that a poorly perfused placenta releases humoral factor(s) that bring(s) about endothelial cell activation in the placental and maternal vascular bed, leading to the onset of pre-eclampsia (Roberts *et al.* 1989). In normal pregnancy maternal decidual arteries undergo vasodilatation to increase blood flow to the intervillous space (Craven *et al.* 1998). Immunohistochemical studies have confirmed that initial stages of these alterations occur in the absence of cellular interaction with extravillous cytotrophoblasts as a maternal response to pregnancy (Craven *et al.* 1998). Later, placental cytotrophoblasts invade the uterus and

undergo transformation of their phenotype to acquire endothelial cell characteristics, which could be critical to endovascular invasion (Zhou *et al.* 1997).

In pregnancies doomed to become pre-eclamptic, trophoblastic invasion does not occur normally. Thus, increased vascular resistance and reduced placental perfusion ensue, which are important early features of pre-eclampsia (Roberts 1998). A primary defect may be a failure of trophoblasts to obtain an endothelial phenotype (Zhou *et al.* 1997). On the other hand, the reduced placental perfusion may also be a consequence of excessive placental size, as in multiple gestation (Ros *et al.* 1998), or of hydropic placentas and hydatidiform moles (Gifford *et al.* 1990), which conditions are often accompanied by an increased risk of pre-eclampsia (Ness and Roberts 1996). Some chromosomal abnormalities such as trisomy 13 (Tuohy and James 1992), or triploidy (Rijhsinghani *et al.* 1997) also increase the risk of pre-eclampsia.

Endothelial dysfunction has been considered central in the pathophysiology of pre-eclampsia (Roberts *et al.* 1989). In this regard, placental cytokines, lipid peroxides, syncytiotrophoblast microvesicles and fluid shear stress, which activate endothelial cell function, may be crucial (Roberts 1998). Cultured villous explants from the human placenta incubated in hypoxic conditions increase the production of immunoreactive cytokines, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 (IL-1) (Benyo *et al.* 1997). Indeed, plasma levels of TNF- $\alpha$  and IL-6 are elevated in pre-eclampsia (Kupferminc *et al.* 1994, Conrad *et al.* 1998). It is also known that the placenta is rich in polyunsaturated fatty acids (Ogburn *et al.* 1988), and thus could serve as a source of lipid peroxides, which are secreted into the maternal circulation (Walsh and Wang 1993). Lipid peroxidation is increased, and the activity of antioxidant enzymes is decreased in pre-eclamptic placentae (Poranen *et al.* 1996). Lipid peroxides damage endothelial cells, inhibit the synthesis of prostacyclin (PGI<sub>2</sub>) and increase the production of thromboxane (TxA<sub>2</sub>), which is a potent vasoconstrictor and stimulator of platelet aggregation (Mäkilä *et al.* 1984, Walsh 1998). Furthermore, the production of vasoconstrictive endothelin (ET)-1 is increased in pre-eclampsia (Taylor *et al.* 1990, Ranta *et al.* 1999), whereas the role of vasodilatory nitric oxide in pre-eclampsia is still controversial (Ranta *et al.* 1998).

### 1.2.2 Maternal factors

Although placental hypoperfusion and other placental factors evidently are important components in the aetiology of pre-eclampsia, they cannot alone account for the disease. This is supported, for example, by the fact that only 30% of infants of pre-eclamptic women are growth-retarded (Eskenazi *et al.* 1993). On the other hand, pre-eclampsia-like trophoblastic changes are also seen in pregnancies complicated by intrauterine growth retardation without pre-eclampsia (Khong *et al.* 1995). There are indeed several maternal factors which clearly predispose to pre-eclampsia (Table 3). These factors, e.g. dyslipidaemia in diabetes and renal changes in hypertension, may also have direct effects on endothelial cells (Ness and Roberts 1996). Hyperinsulinaemia and deficiency of vasodilatory PGI<sub>2</sub> are present in hypertensive pregnant women (Kaaja *et al.* 1995). It is also known that hyperlipidaemic sera from pre-eclamptic women induce triglyceride accumulation in cultured endothelial cells and reduce PGI<sub>2</sub> release (Lorentzen *et al.* 1991). Moreover, the higher ratio of free fatty acids (FFAs) to albumin and increased lipolytic activity in sera from pre-eclamptic women result in enhanced endothelial uptake of FFAs (Endresen *et al.* 1992).

**Table 3. Maternal factors predisposing to pre-eclampsia, Odds ratio (95% confidence interval) or relative risk**

Family history of pre-eclampsia	2- to 5-fold	(Chesley and Cooper 1986)
Collagen vascular disease	3-4-fold	(Kaaja <i>et al.</i> 1990)
Migraine	2.4 (1.4-4.2)	(Marcoux <i>et al.</i> 1992)
Gestational diabetes mellitus	3-fold	(Suhonen and Teramo 1993)
Hyperthyroidism (uncontrolled)	4.7 (1.1-19.7)	(Millar <i>et al.</i> 1994)
Chronic hypertension	9-fold	(Rey and Couturier 1994)
Primiparity	3.8 (2.8-5.2)	(Mittendorf <i>et al.</i> 1996)
Elevated systolic blood pressure (during early pregnancy)	2.7 (1.7-4.3)	(Sibai <i>et al.</i> 1997)
Elevated diastolic blood pressure (during early pregnancy)	1.7 (1.3-2.2)	(Sibai <i>et al.</i> 1997)
Polycystic ovary syndrome	5-6-fold	(de Vries <i>et al.</i> 1998)
Renal disease	7.2 (4.2-12.5)	(Fink <i>et al.</i> 1998)
Insulin- dependent diabetes mellitus	5.6 (2.7-11.4)	(Ros <i>et al.</i> 1998)
Obesity	5.2 (2.4-11.5)	(Ros <i>et al.</i> 1998)
Maternal low birth weight	5.2 (1.2-21.5)	(Innes <i>et al.</i> 1999)
Maternal preterm birth	3.6 (1.3-10.3)	(Innes <i>et al.</i> 1999)

### 1.2.3 Familial tendency

A familial tendency in pre-eclampsia is well-established (Cooper *et al.* 1993). This information dates back to a classic study by Chesley and Cooper (1986), who found pre-eclampsia in 26% of the daughters and 16% of the granddaughters of eclamptic mothers (Chesley and Cooper 1986). Major dominant gene model with reduced penetrance or multifactorial inheritance have been considered the best working hypotheses (Arngrimsson *et al.* 1995). However, although a twin study failed to detect concordant monozygotic twins (Thornton and Onwude 1991), the reduced penetrance model, in which the fetal genotype modifies expression of the maternal genotype, could explain the existence of discordant monozygotic twins (Harrison *et al.* 1997).

At present several groups are conducting linkage studies and complete maternal genome-wide scans in order to discover pre-eclampsia genes. However, so far no such genes have been identified (Harrison *et al.* 1997). The hunt for genes for pre-eclampsia will yield many that may operate as risk factors (Redman *et al.* 1999). An increased risk of pre-eclampsia has been associated with certain polymorphisms and mutations, e.g. in the angiotensinogen gene (Ward *et al.* 1993), in the factor V gene (Lindoff *et al.* 1997), and in the methylenetetrahydrofolate reductase gene (Sohda *et al.* 1997). Polymorphisms and mutations in these diverse genes may be related to pre-eclampsia, because all of them are cardiovascular risk factors. It is obvious that the list of gene mutations thought to be of significance in the aetiology of pre-eclampsia will expand greatly in future.

## 2. Insulin resistance and insulin resistance syndrome

Insulin resistance is defined as an impaired ability of insulin to stimulate the uptake and disposal of glucose by muscle (Reaven *et al.* 1996). Reaven was the first to describe so-called Syndrome X (insulin resistance syndrome) which denotes a cluster of decreased insulin sensitivity, glucose intolerance, hyperinsulinaemia, increased very low-density lipoprotein (VLDL) triglyceride, decreased HDL cholesterol, and hypertension (Reaven 1988). Later on, other conditions, such as hyperuricaemia (Facchini *et al.* 1991), a high plasminogen activator inhibitor-1 level (Juhan-Vague *et al.* 1991, Vuorinen-Markkola and Yki-Järvinen 1994), small, dense low-density lipoprotein (LDL) particles (Reaven *et al.* 1993, Selby *et al.* 1993),

obesity, particularly upper body obesity (Landsberg 1996), and microalbuminuria (Mykkänen *et al.* 1998) have been added to elements of the insulin resistance syndrome. Insulin resistance is a common phenomenon, occurring in approximately 25% of the general population, and it is associated with an increased risk of CVD (Reaven 1994).

Several genetic and metabolic factors have been linked to insulin resistance (Table 4). Gestational diabetes mellitus (GDM) (Clark *et al.* 1997), hyperleptinaemia (de Courten *et al.* 1997, Leyva *et al.* 1998), and PCOS (Davidson 1995) may also be manifestations of the insulin resistance syndrome. Genes are evidently closely associated with the control of insulin action, as in NIDDM concordance rate estimate for monozygotic twins is 34% in twin pairs (Kaprio *et al.* 1992).

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**Table 4. Causes of insulin resistance (Moller and Flier 1991)**

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**Defects intrinsic to target cells**

*Mutations in the insulin receptor gene*

*Defects in other genes important for insulin action*

**Secondary factors affecting target cells**

*Abnormal physiological states*

Stress (e.g. fever, sepsis)

Fasting or starvation

Uraemia

Cirrhosis

Ketoacidosis

Obesity

Diabetes or hyperglycaemia

*Normal physiological states*

Puberty

Advanced age

Pregnancy

*Specific hormonal or metabolic factors*

Glucocorticoids (e.g. Cushing's syndrome)

Growth hormone (acromegaly)

Catecholamines (e.g. pheochromocytoma)

Glucagon (e.g. glucagonoma)

Thyroid hormone (e.g. thyrotoxicosis)

Hyperinsulinaemia (e.g. insulinoma)

Hyperglycaemia (e.g. diabetes)

Non-esterified fatty acids

Adenosine

Islet amyloid polypeptide (amylin)

*Autoantibodies to insulin receptor*

---

## 2.1 Measurement of insulin sensitivity

In clinical practice the measurement of insulin sensitivity is not easy. Increased insulin concentrations in peripheral blood generally reflect insulin sensitivity in non-diabetic subjects (Laakso 1993). However, insulin levels also depend on secretion, distribution, and degradation of insulin in addition to tissue insulin sensitivity (Ferrannini and Mari 1998). The measurement of insulin sensitivity by use of hyperinsulinaemic euglycaemic clamp, the insulin suppression test, or the frequently sampled intravenous glucose tolerance test (the Minimal Model approach) are suitable for study purposes but not for clinical practice as they are expensive and have limited patient acceptance (Haffner and Miettinen 1997).

**The euglycaemic hyperinsulinaemic clamp**, the best standard for the measurement of insulin action, involves a constant insulin infusion while maintaining euglycaemia by infusing a variable amount of glucose (DeFronzo *et al.* 1979). The glucose infusion rate provides a quantitative assessment of the biological effect of insulin. The clamp can be combined with a number of other procedures when studying non-pregnant subjects (Ferrannini and Mari 1998). It requires two intravenous lines, on-line plasma glucose determination, and arterialized blood, commonly accomplished by retrograde cannulation of a heated wrist or hand vein (Ferrannini and Mari 1998). At the end of the clamp study, the subject's plasma glucose must be monitored for some time because the hypoglycaemic effect extends beyond the return of plasma insulin to its baseline value (Ferrannini and Mari 1998).

**The insulin suppression test** is a reverse clamp, in which exogenous glucose infusion is kept constant while the plasma glucose concentration is allowed to vary: at a steady state, the higher the level of blood glucose, the worse the insulin sensitivity (Ferrannini and Mari 1998). Because hyperglycaemia will stimulate endogenous insulin release, the  $\beta$ -cell response must be suppressed by means of somatostatin (Harano *et al.* 1977). In this method some problems may arise: plasma glucose may not stabilize satisfactorily over the infusion period, steady state plasma glucose in insulin-resistant individuals may exceed the renal threshold leading to glucosuria, and somatostatin may inhibit the secretion of hormones other than somatostatin (Ferrannini and Mari 1998).

In the **Minimal Model** an intravenous glucose injection is followed by frequent determinations of plasma glucose and insulin (Bergman 1989). A computer program

allows calculation of insulin sensitivity from the dynamic relationships between the plasma glucose and insulin concentration curves. Minimal Model analysis of frequently sampled intravenous glucose tolerance test requires a discrete insulin response, and therefore the protocol includes an exogenous infusion of insulin 20 minutes after the injection of the glucose bolus (Saad *et al.* 1994). In severe insulin resistance the minimal model may yield negative values for insulin sensitivity (Saad *et al.* 1994). In addition, it has some drawbacks: a need for two intravenous lines, frequent blood sampling, and a risk of hypoglycaemia, which might occur late in the test. Moreover, this method demands the investigator's confidence in a 'black box' of calculations (Ferrannini and Mari 1998). Nevertheless, the Minimal Model is regarded as a representative model, because it gives comparable results with those obtained with the clamp method (Saad *et al.* 1994).

## **2.2 Implications of insulin resistance**

As many as 50% of non-pregnant patients with essential hypertension appear to be insulin resistant and hyperinsulinaemic (Zavaroni *et al.* 1992). Hypertensive patients have enhanced plasma glucose and insulin responses to an oral glucose challenge (Shen *et al.* 1988). The higher plasma concentrations of glucose and insulin in these patients result from insulin resistance in peripheral tissues (Ferrannini *et al.* 1987). Increased sympathetic tone, present in approximately 30% of patients with hypertension, is closely associated with the insulin resistance syndrome, and could be the primary factor of the syndrome (Julius 1998). The significance of insulin resistance and hyperinsulinaemia preceding the onset of hypertension has also been questioned, e.g. in view of the absence of hypertension in a number of populations with other elements of insulin resistance syndrome (Mark and Anderson 1995).

Dyslipidaemia in insulin resistance comprises elevated plasma triglycerides, decreased HDL cholesterol, compositional changes in HDL subclasses, preponderance of small, dense LDL cholesterol, and enhanced postprandial lipaemia (Taskinen 1995). The failure of insulin to suppress VLDL production in the liver may be the central perturbation that leads to the elevation of circulating triglyceride particles in insulin-resistant states (Frayn 1993, Sparks and Sparks 1993). The increased flux of FFAs to the liver is an important contributing factor indicating that



the release of FFAs from triglyceride stores is not adequately suppressed by insulin (Björntorp 1994).

Obesity is characterised by elevated FFA concentrations and enhanced lipid oxidation (Groop *et al.* 1992). Even small increments in body mass index (BMI) and waist-to-hip ratio, as often seen in hypertension, impair insulin sensitivity, probably through altered lipid metabolism (Toft *et al.* 1998). Obesity, particularly visceral adiposity with increased FFA flux into the portal circulation, is associated with insulin resistance (DeFronzo and Ferrannini 1991, Karter *et al.* 1996). Visceral obesity has a stronger association to metabolic and cardiovascular disease than total weight (Kaplan 1989). Women with upper body obesity also have higher androgen production rates and higher circulating free testosterone and free oestradiol concentrations (Kirschner *et al.* 1990), which may be a consequence of insulin-induced promotion of ovarian androgen secretion (Pasquali *et al.* 1993). Insulin resistance precedes NIDDM, but it is not known whether it reflects the strongest risk factor or whether it unmasks a primary  $\beta$ -cell defect (Yki-Järvinen 1994).

In late 1970s the result of three epidemiological prospective studies indicated that hyperinsulinaemia predicts the development of symptomatic CVD in men (Welborn and Wearne 1979, Ducimetiere *et al.* 1980, Pyörälä *et al.* 1985). An association between hyperinsulinaemia and CVD in women was reported in 1998 (Bonora *et al.* 1998). The mechanism(s) by which the insulin resistance syndrome and hyperinsulinaemia may cause CVD may be related to endothelial dysfunction and disturbances in coagulation. Insulin resistance is associated with impaired fibrinolysis (Juhan-Vague *et al.* 1991), blunted endothelium-dependent vasodilatation (Steinberg *et al.* 1996), reduced PGI<sub>2</sub> production, increased TXA<sub>2</sub> production, increased coagulation, and platelet activation (McCarty 1995). Acute-phase cytokines, such as IL-6 and TNF- $\alpha$  could underlie the associations of insulin resistance with coagulopathy, endothelial dysfunction, and CVD (Yudkin 1999).

Endothelial dysfunction might also precede insulin resistance. This is supported by the fact that endothelial injury may cause a loss of glycosaminoglycan and lipoprotein lipase (LPL, an enzyme hydrolyzing triglycerides) at the endothelial surface (Pinkney *et al.* 1997). Dysfunction of LPL has been shown to lead to increased plasma triglyceride concentrations and reduced HDL cholesterol (Reymer *et al.* 1995). HDL cholesterol may stimulate endothelial cell function, e.g. by stimulating PGI<sub>2</sub>-release from vascular endothelium (Pomerantz *et al.* 1985,

Tamagaki *et al.* 1996). This hypothesis may explain why the dyslipidaemia of insulin resistance is confined to triglycerides and HDL.

### 3. Carbohydrate metabolism in pregnancy

#### 3.1 Normal pregnancy

The levels of fasting glucose decrease, and those of fasting insulin increase in late gestation (Catalano *et al.* 1992). However, hepatic insulin sensitivity in late pregnancy is decreased, as evidenced by a significant (30%) increase in basal hepatic glucose production in late gestation (Catalano 1994). An increase in maternal plasma volume and facilitated transport of glucose from mother to fetus may explain the decreases in fasting glucose concentrations (Kalhan *et al.* 1979, Catalano 1994). The insulin response to glucose may be 3.0-3.5 times higher at 34-36 weeks' gestation than before pregnancy (Catalano *et al.* 1991). Peripheral insulin sensitivity in late pregnancy is 33-66% decreased in comparison with the non-pregnant state (Ryan *et al.* 1985, Buchanan *et al.* 1990, Catalano *et al.* 1991). True maternal insulin sensitivity may be even lower than the estimates presented above, because glucose is also transported to the fetus by non-insulin-mediated pathways (Catalano 1994).

The ultimate causes and the physiological meaning of pregnancy-induced insulin resistance are not known, but elevated FFA concentrations may be one contributing factor (Sivan *et al.* 1998). In this regard, a defect in glucose transport or phosphorylation (Boden *et al.* 1994, Hawkins *et al.* 1997) and increased activity of the hexosamine pathway (Hawkins *et al.* 1997) may be significant. Human placental lactogen (HPL), cortisol, and sex steroids, which are also at elevated concentrations in pregnancy, are also likely candidates as regards the regulation of insulin sensitivity (Ryan and Enns 1988), and, for example, HPL stimulates FFA release from adipose tissue (Williams and Coltart 1978).

#### 3.2 Pre-eclampsia

Pre-eclampsia is associated with increased fasting insulin concentrations and higher glucose and insulin responses in the oral glucose tolerance test (OGTT), but the data on insulin sensitivity are contradictory (Table 5). It is also known that an increased glucose response in the OGTT (Lindsay *et al.* 1989, Sermer *et al.* 1995, Joffe *et al.*

1998), and elevated fasting insulin levels (Sowers *et al.* 1995) predict the risk of pre-eclampsia (Table 6).

**Table 5. Glucose and insulin in pre-eclamptic and normal pregnancies before and after glucose loading**

<i>Subjects</i>	<i>Method</i>	<i>Findings</i>	<i>Reference</i>
Pre-eclamptic women, women with gestational hypertension,	Fasting levels of insulin at 21-39 weeks' gestation	Fasting insulin ↑	(Kaaja <i>et al.</i> 1995)
Pre-eclamptic women	Fasting levels of glucose and insulin, 1-hour 50g OGTT at 32 weeks' gestation	Fasting insulin ↑ Post-load insulin ↑	(Martinez Abundis <i>et al.</i> 1996)
Pre-eclamptic women,	Two-hour OGTT at 37 weeks' gestation	Fasting glucose ↓ Post-load glucose ↑ Post-load insulin ↑	(Lorentzen <i>et al.</i> 1998)
Pre-eclamptic women	The Minimal Model	Insulin sensitivity ↑	(Roberts <i>et al.</i> 1998)
Women with pre-eclampsia, gestational hypertension (GH), and chronic hypertension (CH)	The euglycaemic hyperinsulinaemic clamp	Insulin sensitivity ↓ in GH, but not in pre-eclampsia or CH	(Caruso <i>et al.</i> 1999)

## 4. Lipids and lipoproteins in pregnancy

### 4.1 Normal pregnancy

Normal pregnancy is characterised by progressive increases in triglyceride (300%) and cholesterol (50%) concentrations (Potter and Nestel 1979). In the late second trimester increased amounts of FFAs are released into the circulation through combined stimulation of hormone-sensitive lipase by HPL and by increased insulin resistance (Sattar *et al.* 1996). Oestrogen increases hepatic output of VLDL, and decreases hepatic lipase activity promoting accumulation of triglycerides in lipoproteins of densities higher than that of VLDL (Alvarez *et al.* 1996). Decreased adipose tissue LPL activity and decreased hepatic lipase activity impair the removal of triglyceride-rich lipoproteins from the circulation (Alvarez *et al.* 1996), thus leading to higher serum triglyceride concentrations.

<b>Table 6. Impaired glucose tolerance predicts the risk of pre-eclampsia</b>			
<i>Subjects</i>	<i>Method</i>	<i>Findings</i>	<i>Reference</i>
Women with one abnormal OGTT value	1-hour 50g OGTT, 3-hour 100g OGTT at 24-32 weeks' gestation	Elevated risk of pre-eclampsia associated with one abnormal OGTT value vs. controls [OR 2.81 (1.26-6.28)]	(Lindsay et al. 1989)
Women with GDM, and one abnormal OGTT value	2-hour 75g OGTT at 28-32 weeks' gestation	Elevated risk (3-fold) for pre-eclampsia in women with GDM, but not in women with one abnormal OGTT value	(Suhonen and Teramo 1993)
Pre-eclamptic women Women with gestational hypertension	1-hour 50g OGTT at 24-32 weeks' gestation	A 1-hour 50g OGTT value $\geq 7.8$ mmol/L predicted pre-eclampsia, but not after adjustment for age, race, gestational age at OGTT and pregravid BMI	(Solomon et al. 1994)
Women without GDM	1-hour 50g OGTT, 3-hour 100g OGTT at 26-28 weeks' gestation	The 2-hour 100g OGTT value was a significant predictor of pre-eclampsia after adjusting for BMI	(Sermer et al. 1995)
Nulliparous women	Fasting levels of glucose and insulin at 18-22 weeks' gestation	Fasting levels of insulin 1.8-fold higher in women who developed pre-eclampsia	(Sowers et al. 1995)
Nulliparous women with GDM	1-hour 50g OGTT, 3-hour 100g OGTT in the late II trimester	The 1-hour 50g OGTT value predicted pre-eclampsia even in normoglycaemic women	(Joffe et al. 1998)

## 4.2 Pre-eclampsia

In pre-eclampsia, triglyceride and FFA levels are markedly increased (Lorentzen *et al.* 1995, Hubel *et al.* 1996, Murai *et al.* 1997), whereas total cholesterol and LDL levels are normal, and HDL concentrations decreased (Sattar *et al.* 1997). Elevations in serum triglyceride and FFA levels are already present before 20 weeks' gestation in women with future pre-eclampsia (Lorentzen *et al.* 1994). In addition, the concentrations of triglyceride-rich lipoproteins and of small dense LDL particles are elevated in pre-eclampsia (Sattar *et al.* 1997). Furthermore it is known that antibodies to oxidized LDL are increased in pre-eclampsia (Branch *et al.* 1994), but they fail to predict the risk of this disease (Kurki *et al.* 1996). It is likely that increased hepatic lipase activity in pre-eclampsia is responsible for the reduced HDL levels (Sattar *et al.* 1997). Thus, pre-eclampsia, manifesting largely in the vascular bed in

pregnancy is accompanied by a number of changes in lipid and lipoprotein concentrations.

Increased plasma cytokine (IL-1, TNF- $\alpha$ ) concentrations in pre-eclampsia resulting from activation of macrophages/neutrophils may enhance peripheral lipolysis (Sattar *et al.* 1996). This may increase the flux of FFAs in the liver, and increase hepatic secretion of large, triglyceride-rich VLDL particles (Sattar *et al.* 1996). Free fatty acids entering the liver are normally either subjected to  $\beta$ -oxidation or secreted as triglycerides in VLDL. An increased frequency of pre-eclampsia has been observed in association with a  $\beta$ -oxidation defect, as is the case in long-chain 3-hydroxyacyl-coenzyme A deficiency (Tyni *et al.* 1998). The failure of insulin to suppress VLDL production in the liver may also be a central perturbation in pre-eclampsia leading to higher serum triglycerides.

## 5. Leptin

### 5.1 Leptin in a non-pregnant state

Leptin is produced almost exclusively by adipocytes (Zhang *et al.* 1994). It takes a hormonal signal to the brain concerning the adequacy of energy stores, and appears to activate the hypothalamic centres regulating energy intake and expenditure (Mantzoros and Moschos 1998). Serum leptin concentrations correlate highly with percentage of body fat (Considine *et al.* 1996). Gender has a major influence on leptin levels. Women have higher plasma leptin levels than men at any degree of adiposity (Saad *et al.* 1997). Several pieces of evidence imply that insulin increases leptin secretion (Kolaczynski *et al.* 1996, Malmström *et al.* 1996, Utriainen *et al.* 1996, Boden *et al.* 1997, Saad *et al.* 1998). Hence, in insulin resistance, independent of adiposity, plasma leptin concentrations are elevated (Segal *et al.* 1996, Haffner *et al.* 1997). The evidence is so strong that hyperleptinaemia has been suggested to be part of the insulin resistance syndrome (de Courten *et al.* 1997). Leptin-stimulated angiogenesis may also facilitate lipid release from fat stores to maintain energy homeostasis (Sierra-Honigmann *et al.* 1998).

### 5.2 Leptin in pregnancy

Circulating leptin levels increase by some 100 to 200% during normal pregnancy, reaching peak levels between 20 and 30 weeks of gestation (Sattar *et al.* 1998, Sivan

*et al.* 1998). These elevations may be derived from adipocytes, but placental trophoblasts must also be taken into account as a source, since they produce leptin (Masuzaki *et al.* 1997, Mise *et al.* 1998). The relative contribution of these cells to the circulating leptin pool in pregnancy is as yet unknown. Leptin correlate with estradiol and human chorionic gonadotrophin levels (Hardie *et al.* 1997). The physiological role of leptin in pregnancy is unknown, but it may help to guarantee adequate circulating FFAs and glucose for the fetus (McCarthy *et al.* 1999).

## 6. Homocysteine

### 6.1 Homocysteine in a non-pregnant state

Homocysteine is a sulphur-containing amino acid formed from methionine (Welch and Loscalzo 1998). This amino acid has attracted much interest, because even moderately elevated levels of homocysteine appear to predict CVD in men and non-pregnant women (Stampfer *et al.* 1992, Graham *et al.* 1997). However, this was not observed in the North Karelia Project (Alfthan *et al.* 1994). Hyperhomocysteinaemia may damage vascular health through endothelial dysfunction, platelet activation, procoagulation, and increased lipid peroxidation (Welch and Loscalzo 1998). Insulin resistance and hyperhomocysteinaemia may coexist in apparently healthy, non-obese subjects (Giltay *et al.* 1998). Patients with NIDDM have higher homocysteine levels than non-diabetic control subjects (Chico *et al.* 1998). A role for insulin resistance in homocysteine metabolism is indicated by decreased plasma homocysteine levels during acute hyperinsulinaemia (hyperinsulinaemic euglycaemic clamp) in healthy subjects but not in patients with NIDDM (Fonsesca *et al.* 1998).

### 6.2 Homocysteine in pregnancy

Normal pregnancy is associated with decreased homocysteine levels (Andersson *et al.* 1992), but in pre-eclamptic pregnancies such falls in homocysteine levels do not occur (Rajkovic *et al.* 1997, Powers *et al.* 1998). In pre-eclampsia, plasma homocysteine concentrations are in correlation with those of plasma fibronectin, a major cell-surface glycoprotein in endothelial cells, suggesting a role for homocysteine in endothelial cell damage (Powers *et al.* 1998).

Elevated homocysteine levels have been reported in women with a history of pre-eclampsia (Dekker *et al.* 1995), placental abruption or infarction (Goddijn-Wessel *et*

*al.* 1996), unexplained recurrent pregnancy loss (Wouters *et al.* 1993), and in those who have given birth to infants with a neural tube defect in previous pregnancies (Stegers-Theunissen *et al.* 1994).

## 7. Activin A and inhibin A in pregnancy

The dimeric glycoproteins activin A and inhibin A, which were originally isolated from ovarian follicular fluid (Ying 1988), are also produced by the decidua, placenta, and fetal membranes (Qu and Thomas 1995). Circulating levels of activin A and inhibin A thus rise with advancing gestational age (Petraglia *et al.* 1993, Muttukrishna *et al.* 1995). During pregnancy, concomitantly with activin A and inhibin A, the serum levels of pro- $\alpha$ C inhibin also rise (Muttukrishna *et al.* 1997). Serum concentrations of activin A and inhibin A are increased in established pre-eclampsia (Muttukrishna *et al.* 1997), but the predictive value of maternal mid-pregnancy serum levels of inhibin A in pre-eclampsia is controversial (Cuckle *et al.* 1998, Rätty *et al.* 1999).

Elevated levels of activin A in gestational diabetes (Petraglia *et al.* 1995) and elevated levels of inhibin A in pregnant women with insulin-dependent diabetes (Wallace *et al.* 1997), as well as the capacity of activin A to increase insulin secretion in incubated (Verspohl *et al.* 1993) or perfused (Yasuda *et al.* 1993, Furukawa *et al.* 1995) rat pancreatic islets and in insulinoma cell lines (Shibata *et al.* 1996) implies a connection between glucose homeostasis, activin A and inhibin A.

## 8. The metabolic effects of antihypertensive drugs

Antihypertensive drugs have different effects on insulin sensitivity. Calcium channel blockers and angiotensin-converting enzyme inhibitors are neutral in this regard, whereas treatment with  $\beta$ -blockers or diuretics is associated with impaired insulin sensitivity (Lithell 1997). Diuretics are seldom used in pre-eclampsia, because they may aggravate hypovolaemia and reduce placental perfusion (Lowe and Rubin 1992). Angiotensin-converting enzyme inhibitors are contraindicated during pregnancy, because they are associated with congenital malformations, neonatal renal failure, intrauterine death, and reduced placental perfusion (Lowe and Rubin 1992).

Isradipine is a dihydropyridine calcium antagonist with a high affinity for slow calcium channels. It produces arterial vasodilatation, but does not affect cardiac

filling pressures (Brogden and Sorkin 1995). It has been used for treatment of hypertension in men and non-pregnant women for years. It has also been used for the control of hypertension in pregnancy, and, indeed, oral administration of isradipine at 2.5-5.0 mg twice daily has been shown to decrease blood pressure without any deterioration in uteroplacental perfusion (Feiks *et al.* 1991). Isradipine has no effect on glucose tolerance, insulin secretion or insulin action in non-pregnant hypertensive patients with (Klauser *et al.* 1991) or without NIDDM (Lind *et al.* 1994). On the other hand, isradipine elevates HDL levels (Brogden and Sorkin 1995). The effects of isradipine on carbohydrate and lipid metabolism in pre-eclampsia are unknown.

Metoprolol is an effective antihypertensive agent that selectively blocks  $\beta_1$ -receptors (Frishman and Chesner 1988). Metoprolol, as do all  $\beta$ -blockers, reduces insulin sensitivity in patients with essential hypertension (Suter and Vetter 1995), and this reduction can be up to 15% (Lithell 1997). Metoprolol and other  $\beta_1$ -blockers can also raise triglyceride levels and lower HDL cholesterol (Suter and Vetter 1995). No data exist on the effects of metoprolol on lipids, lipoproteins or insulin sensitivity in pregnant women.

## 9. Impact of a history of pre-eclampsia on pregnancy outcome and cardiovascular morbidity

### 9.1 Subsequent pregnancies

Women having had pre-eclampsia or eclampsia in their first pregnancies are at an increased risk of a repeat this disease (Table 7), although the disease generally occurs at a greater gestational age (Steegers and van der Post 1998). The recurrence risk of pre-eclampsia is best predicted by the degree of proteinuria. The recurrence risk of pre-eclampsia in women who had had proteinuria of 0.3-3 g/24 hours was 12.2% compared with 22.3% in women who had had proteinuria of  $\geq 3$  g/24 hours in their index pregnancy (Visser *et al.* 1999). In general, estimation of the risk ratio is difficult, because most of the studies lack controls, and the incidence of eclampsia and pre-eclampsia may be different in different populations.



**Table 7. The recurrence rate of pre-eclampsia/eclampsia in women with a history of prior pre-eclampsia/eclampsia**

Country	Study population	%	Reference
Mexico	110 eclamptic women	35.4%	(Lopez-Llera and Horta 1974)
U.K.	279 pre-eclamptic women (3507 normotensive women)	7.4% 0.7%	(Campbell <i>et al.</i> 1985)
Nigeria	64 eclamptic women	26.6%	(Adelusi and Ojenbede 1986)
USA	406 pre-eclamptic women (409 normotensive women)	46.8% 7.6%	(Sibai <i>et al.</i> 1986)
USA	89 pre-eclamptic women	55%	(Sibai <i>et al.</i> 1991)
USA	159 eclamptic women	24%	(Sibai <i>et al.</i> 1992)
USA	606 pre-eclamptic women		(Caritis <i>et al.</i> 1998)
	aspirin group	17%	
	placebo group	19%	

## 9.2 Cardiovascular morbidity

Conflicting data exist on the impact of pre-eclampsia on the risk of future hypertension. Some researchers report that eclampsia and pre-eclampsia do not predispose women to hypertension in later life (Chesley 1980, Fisher *et al.* 1981), while others have found an increased incidence of hypertension, particularly in women with recurrent pre-eclampsia (Sibai *et al.* 1991, Sibai *et al.* 1992). The incidence of hypertension has been reported to be significantly higher after 7 to 8 years (range 2 to 24 years) of follow-up in women having had pre-eclampsia in their first pregnancy (14.8%) than in women with normotensive first pregnancy (5.6%) (Sibai *et al.* 1986). The difference increased in a subgroup followed for over 10 years (51% in women with prior pre-eclamptic pregnancy vs. 14% in women with prior normotensive pregnancy) (Sibai *et al.* 1986).

After 25 years of follow-up, 29% of deaths in women having had eclamptic first pregnancy have been reported to be due to CVD, whereas in women having had eclampsia as multiparas 82% of deaths were of cardiovascular-renal origin (Chesley *et al.* 1976). In Iceland the relative risk of dying from subsequent CVD was higher among women with prior eclampsia (RR 2.61; 95% CI 1.11-6.12) and among those with prior pre-eclampsia (RR 1.90; 95% CI 1.02-3.52) than among those with prior hypertensive pregnancy alone (Jónsdóttir *et al.* 1995). Parous women at the index pregnancy had a twofold higher risk of dying from CVD than primigravid women (RR

2.05; 95% CI 1.19-3.55) (Jónsdóttir *et al.* 1995). These two studies imply that a history of pre-eclamptic or hypertensive pregnancy indicated an increased risk of CVD primarily in multiparous women. Similar data have been reported in the UK, where the risk of hypertensive disease (RR 2.35; 95% CI 2.08-2.65), acute myocardial infarction (RR 2.24; 95% CI 1.42-3.53), chronic ischaemic heart disease (RR 1.74; 95% CI 1.06-2.86), angina pectoris (RR 1.53; 95% CI 1.09-2.15), and venous thromboembolism (RR 1.62) were markedly elevated in women who had had pre-eclampsia (Hannaford *et al.* 1997).

The mechanism behind the pre-eclampsia-associated impact on CVD is open. However, decreased insulin sensitivity, as measured in Chinese women with a history of pre-eclampsia two months postpartum by the insulin suppression test (Fuh *et al.* 1995), suggested that insulin resistance could be a factor. In another study in African-American women 3-6 months postpartum, insulin sensitivity, as assessed by the hyperinsulinaemic-euglycaemic clamp technique, was not different between women with prior pre-eclampsia and controls (Jacobson *et al.* 1994). Different race or different methods to assess insulin sensitivity may explain the discrepant data.

The incidence of diabetes in women with prior pre-eclamptic pregnancy (5.6%) is also higher than in controls (3.6%) (Sibai *et al.* 1986). This was also seen in another study in both primiparous (2.5-fold) and multiparous (3.3-fold) eclamptic women after 25-year follow-up (Chesley 1976). These data also may suggest a link between insulin resistance and pre-eclampsia.

### III AIMS OF THE STUDY

The present studies were undertaken to investigate insulin sensitivity and its relationships to leptin, homocysteine and activin-inhibin in pre-eclampsia. The specific aims were:

To compare insulin sensitivity and lipids and lipoproteins in pre-eclamptic and healthy pregnant women during and after pregnancy

To compare the circulating levels of leptin, homocysteine, activin A and inhibin A in pre-eclamptic and healthy pregnant women, both during and after pregnancy, and to assess whether they are associated with insulin sensitivity

To study whether isradipine or metoprolol affect insulin sensitivity and lipid profiles in pre-eclamptic women

To compare glucose tolerance, lipid profiles, some sex hormone levels and endothelial function in women with a prior pre-eclamptic pregnancy, and in women with a prior normotensive pregnancy

## IV SUBJECTS AND METHODS

### 1. Subjects

This study was performed with the approval of the Ethics Committee of the Department of Obstetrics and Gynaecology, University of Helsinki. All subjects gave their consent after being informed of the purpose, nature and possible risks of the studies. Pre-eclampsia was defined as a blood pressure level  $\geq 140/90$  mmHg confirmed by at least two blood pressure measurements six hours apart and proteinuria  $\geq 0.3$  g/24 h urine collection between 29-39 weeks of gestation (Gifford *et al.* 1990). Severe pre-eclampsia was defined as blood pressure  $\geq 160/110$  mmHg, and proteinuria  $\geq 0.3$  g/24 h. Altogether, 91 subjects were studied: 8 patients participated in both studies I-IV and V (Table 8).

**Table 8. Clinical characteristics of the study population (mean $\pm$ SE)**

	Pre-eclamptic women			Controls		
	No. of women	Age (years)	BMI (kg/m <sup>2</sup> )	No. of women	Age (years)	BMI (kg/m <sup>2</sup> )
<b>Studies I-IV</b>						
-during pregnancy	22	30.4 $\pm$ 1.0	22.6 $\pm$ 0.5*	16	31.8 $\pm$ 1.1	21.9 $\pm$ 0.8*
-after delivery	14		23.9 $\pm$ 0.8	11		23.5 $\pm$ 1.0
<b>Study V</b>						
-isradipine group	9	29.7 $\pm$ 1.8	23.8 $\pm$ 1.4*			
-metoprolol group	8	31.6 $\pm$ 1.8	22.4 $\pm$ 1.2*			
<b>Studies VI-VII</b>						
-at first pregnancy	22	24.8 $\pm$ 0.9	20.7 $\pm$ 0.6*	22	25.0 $\pm$ 0.9	19.4 $\pm$ 0.3*
-at entry	22	41.8 $\pm$ 0.9	23.3 $\pm$ 0.8	22	41.8 $\pm$ 0.9	21.9 $\pm$ 0.4

\*Pre-pregnancy body mass index (BMI)

Previously healthy, nulliparous pre-eclamptic women and 16 healthy, nulliparous pregnant controls were recruited from maternity clinics, the prenatal clinic and the antenatal ward between January 1, 1996 and February 28, 1998 to studies I-V. Only the pre-eclamptic patients were hospitalised. All women were carrying a single foetus, and no one used antihypertensive medication, aspirin or corticosteroids. Their glucose tolerance was confirmed to be normal by a 75 g oral glucose-tolerance test (fasting:  $\leq 4.5$  mmol/L; at 1 h:  $\leq 9.1$  mmol/L; at 2 h  $\leq 7.9$  mmol/L). None of the controls developed hypertension during the remaining weeks of gestation. In study

V, patients were reinvestigated 5 to 7 days after the initiation of the antihypertensive drug. In studies I-III the study subjects were invited to re-examination on average 12 weeks (range 6-22 weeks) after delivery. In studies VI-VII we investigated women who had given birth to their first child in 1976 to 1978.

To detect 25% difference in insulin sensitivity between the medications used in the study V with 80% power, when type 1 error ( $\alpha$ ) is 0.05, we would have needed 20 patients in each group. This power analysis was also employed when we planned the number of patients and controls in the study I.

Two-hour oral glucose tolerance tests were performed with 75 g glucose after an overnight fast. In studies I-V we measured insulin sensitivity by using a frequently sampled intravenous glucose tolerance test with Minimal Model analysis (Bergman 1989). After an overnight fast a bolus of glucose (0.3 g/kg body weight) was injected intravenously at 0900 hours followed by a bolus of human insulin (Velosulin<sup>R</sup> Human, Novo Nordisk Pharmaceuticals, Denmark, 0.03 IU/kg) 20 min later. Blood samples were collected as follows: 2 at baseline with a 5-minute interval, and 4, 6, 8, 10, 19, 22, 29, 37, 67, 90 and 180 minutes after administration of the glucose bolus. Insulin sensitivity was evaluated from disappearance curves of glucose and insulin, using the Minimal Model computer program. Blood and urine samples were assayed for various agents, using established methods (Table 9)

**Table 9. Characteristics of assays used (CV=intra/interassay coefficient of variation)**

<i>Factor</i>	<i>Source of reagents</i>	<i>Principle of the assay</i>	<i>CV(%)</i>
Activin-A	Serotec Limited, Oxford, UK	Solid-phase sandwich enzyme immunoassay	<10 / ND
Androstenedione	Antiserum from ICN Biomedica Tracer from Amersham	RIA	6.0 / 8.5
C-peptide	Byk-Sangtec Diagnostica, Germany	RIA	3.5 / 3.6
Cholesterol total	Roche, Switzerland	Enzymatic method	< 0.5 / 2.1
Cholesterol HDL	Boehringer Mannheim, Germany	Precipitation of the other lipoproteins with heparin and manganese chloride	4.7 / 5.4
Cholesterol HDL <sub>2</sub>	Boehringer Mannheim, Germany	Precipitation with 0.11% dextran sulphate	<4.0 / <6.0
Cholesterol HDL <sub>3</sub>	Boehringer Mannheim, Germany	Non-precipitable cholesterol	3.0 / 6.8

Cholesterol LDL		Calculated using the Friedewald equation	
DHEAS	Coat-A-Count <sup>R</sup> , Diagnostic Product Corporation, CA, USA	RIA	7.4 / 10.2
2,3 Dinor-6-keto-prostaglandin F1 $\alpha$	Own antibody, tracer from Amersham International	HPLC, RIA	<8 / 10.4-14.1
2,3-Dinor thromboxane B2	Own antibody, tracer from Amersham International	HPLC, RIA	<8 / 10.4-14.1
Endothelin-1	Own antibody, tracer from Amersham International	RIA	5.7
FSH	DELFLIA, Wallac, Turku, Finland	Fluoroimmunoassay	2.9 / 3.9
Free fatty acids	E.Merck, Darmstadt, Germany	Gas chromatograph, methyl ester derivatives	
Glucose	Merck Kebolab, Germany	Amperometric method	- / 2.6
Homocysteine		HPLC fluorescence detector	
IGFBP-1	Medix Biochemica, Kauniainen, Finland	Immunoenzymometric assay	2.9 / 6.8
Inhibin A	Serotec Limited, Oxford, UK	Solid-phase sandwich enzyme immunoassay	<10 / ND
Insulin	Pharmacia, Uppsala, Sweden	RIA	6.8 / 7.7
Leptin	Human leptin RIA kit, Linco Research Inc., St. Louis, MO	RIA	3.8-4.7/-
LH	DELFLIA, Wallac, Turku	Fluoroimmunoassay	1.8/3.4
Oestradiol	Sorin Biomedica Diagnostics, Saluggia Italy	RIA	5.7 / 5.7
Pro- $\alpha$ C	Serotec Limited, Oxford, UK	Solid-phase sandwich enzyme immunoassays	<10 / ND
SHBG	DELFLIA, Wallac, Turku, Finland	Fluoroimmunoassay	1.5 / 4.0
Testosterone	Spectria Testosterone [ <sup>125</sup> I] Coated Tube Radioimmunoassay, Orion Diagnostica, Espoo, Finland	RIA	5.3 / 8.9
Thyroxine	ACS <sup>TM</sup> T4, Ciba Corning	Competitive immunoassay	3.2 / 6.4
Triglycerides	Roche, Switzerland	Enzymatic method	< 0.5 / 2.0
Uric acid	Boehringer Mannheim, Germany	Enzymatic method	1.2 / 2.2

DHEAS = dehydroepiandrosteronesulphate, FSH =follicle-stimulating hormone, LH = luteinizing hormone, IGFBP-1 = insulin growth factor-binding protein-1, RIA = radioimmunoassay, SHBG=sex hormone-binding globulin, HPLC = high performance liquid chromatography

### 3. Statistical analysis

Continuous variables are presented as mean  $\pm$  standard error of the mean (Studies I-III and VI-VII). In study IV glycoprotein results are presented as geometric means and 95% confidence intervals (CIs). In the study V data on metabolic variables, umbilical artery resistance index and BP are presented as means and 95% CIs.

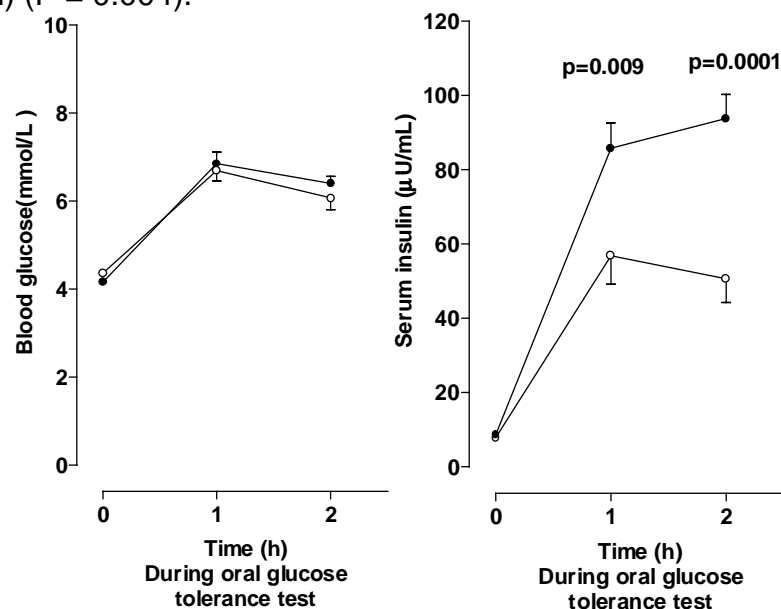
Comparisons between groups were performed with Student's two-tailed unpaired *t* test and within each group with Student's two-tailed paired *t* test for variables which were normally distributed either without or after log transformation (Studies I-VII). Apgar score data were analysed by the Mann-Whitney non-parametric test (Study V). Fisher's exact probability test was used for comparisons between category variables (Study V). Relationships between different variables were investigated by linear and multiple regression analysis (Studies I-VII). Bonferroni corrections were not done for multiple comparisons, and  $P < 0.05$  was considered significant. A linear model taking into account gestational age was used to investigate the relationships of glycoproteins to different variables in study IV. The areas under the response curves for blood glucose and serum insulin were calculated by the trapezoidal rule (Studies I, V and VI). Receiver-operating-characteristic (ROC) curves indicating the diagnostic accuracy of activin A, inhibin A and pro- $\alpha$ C assays in pre-eclampsia were used in study IV. The disposition index, reflecting  $\beta$ -cell function in regard to insulin sensitivity was calculated as insulin secretion (expressed as incremental area under the insulin curve during the first ten minutes) multiplied by insulin sensitivity (Study I). Free testosterone was calculated by using the formula: serum total testosterone in pmol/L / 100 x (2.28-1.38 x logarithm serum sex hormone-binding globulin (SHBG) in nmol/L / 10) (Study VII).

## V RESULTS

### 1. Insulin sensitivity, lipids and lipoproteins during and after pre-eclamptic pregnancy (I)

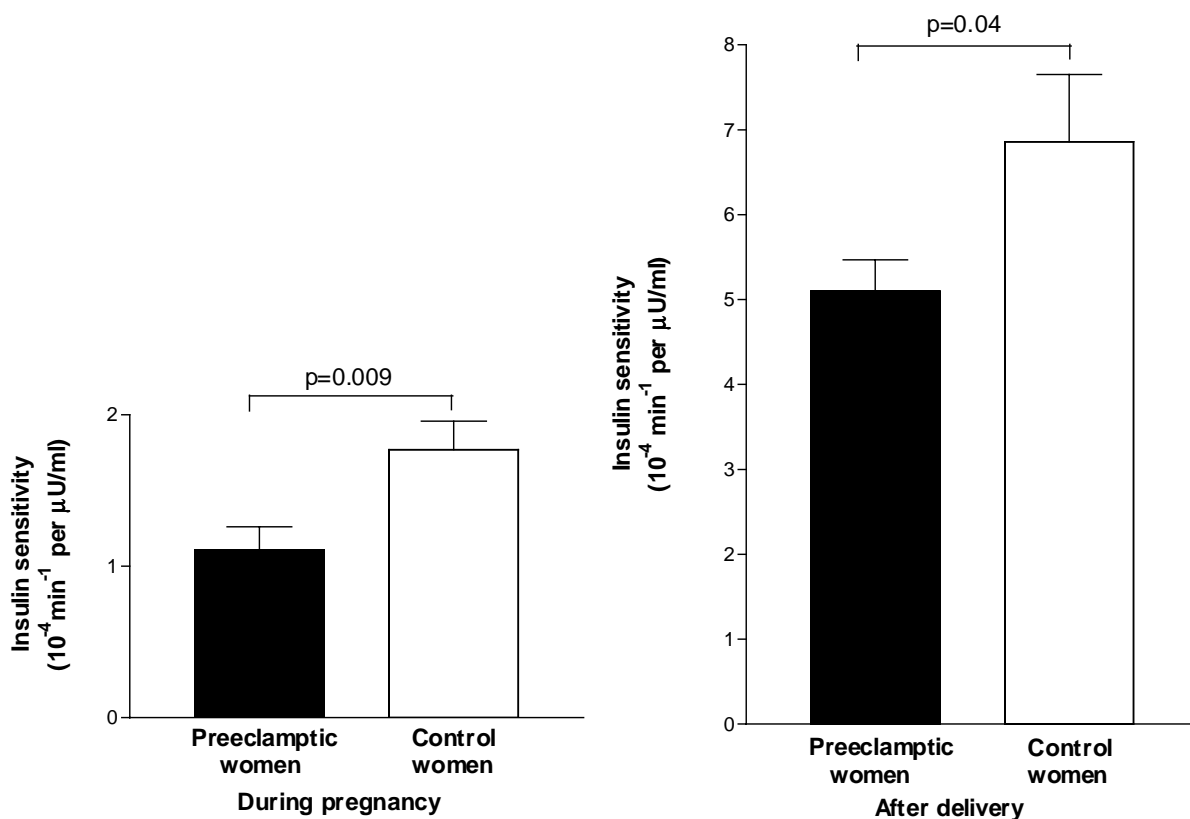
Pre-eclamptic and control women had a similar blood glucose response in the oral glucose tolerance test, but pre-eclamptic women's insulin responses were significantly higher (Figure 1). The area under the insulin curve in pre-eclamptic women ( $8221 \pm 560 \mu\text{U}/\text{mL} \times \text{min}$ ) was 59% larger ( $P = 0.001$ ) than that in the control women ( $5157 \pm 611 \mu\text{U}/\text{mL} \times \text{min}$ ). In contrast to normal basal insulin levels, fasting C-peptide levels in pre-eclamptic women ( $0.73 \pm 0.04 \text{ nmol}/\text{L}$ ) were higher ( $P = 0.03$ ) than in control women ( $0.58 \pm 0.05 \text{ nmol}/\text{L}$ ). The C-peptide/insulin ratio showed no difference between the two groups.

Insulin sensitivity ranged from  $0.26 \times 10^{-4} \text{ min}^{-1} \cdot \text{per } \mu\text{U}/\text{mL}$  to  $2.52 \times 10^{-4} \text{ min}^{-1} \cdot \text{per } \mu\text{U}/\text{mL}$  in pre-eclamptic women, and from  $0.45 \times 10^{-4} \text{ min}^{-1} \cdot \text{per } \mu\text{U}/\text{mL}$  to  $3.62 \times 10^{-4} \text{ min}^{-1} \cdot \text{per } \mu\text{U}/\text{mL}$  in controls. As a mean, insulin sensitivity was 37% lower ( $P = 0.009$ ) in pre-eclamptic women than in controls (Figure 2 and 3). The first 10-minute insulin response, expressed as the area under the curve, was 53% higher in pre-eclamptic women ( $971.0 \pm 81.6 \mu\text{U}/\text{mL} \times \text{min}$ ) than in control women ( $633.8 \pm 61.5 \mu\text{U}/\text{mL} \times \text{min}$ ) ( $P = 0.004$ ).

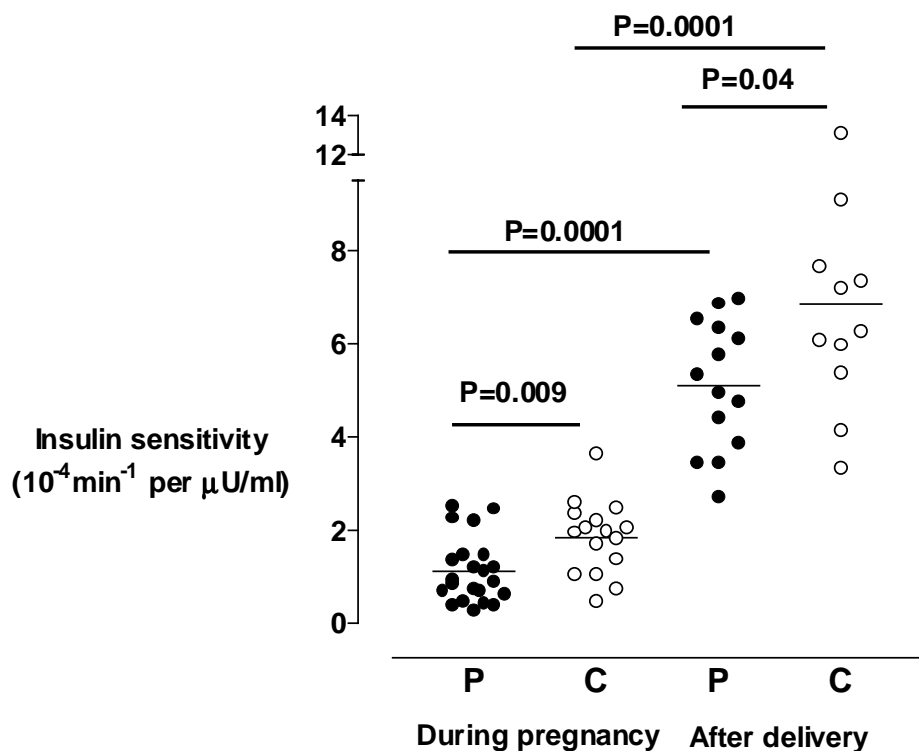


**Fig. 1.** Levels of blood glucose and serum insulin before and following a standard oral glucose dose (75 g) in pre-eclamptic women (●) and normotensive pregnant control women (○). Data are means  $\pm$  SE.





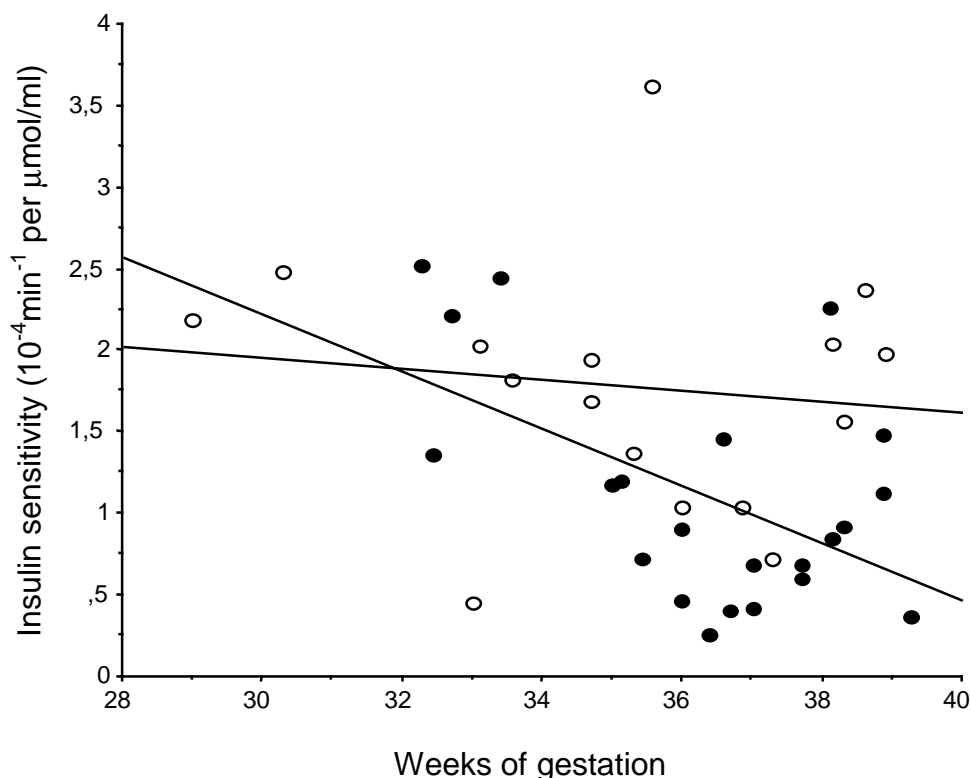
**Fig. 2.** Insulin sensitivity in the Minimal Model in women with pre-eclampsia and in normotensive control women. **A** during pregnancy and **B**, 12 weeks after delivery. Data are means  $\pm$  SE.



**Fig. 3.** Insulin sensitivity as assessed by the Minimal Model during pregnancy and 12 weeks after delivery. P=pre-eclamptic women, C=control women

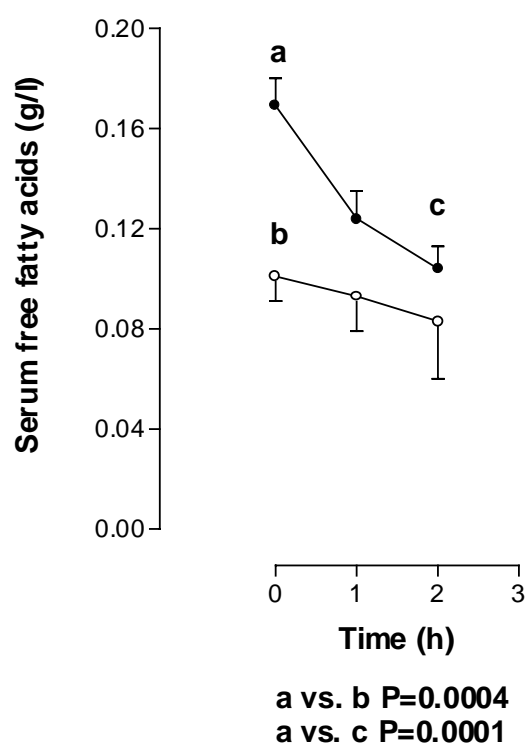
Twelve weeks after delivery, insulin sensitivity had increased 4.6-fold in the pre-eclamptic women (from  $1.11 \pm 0.15$  to  $5.10 \pm 0.37 \times 10^{-4} \text{ min}^{-1} \cdot \text{per } \mu\text{U/mL}$ ,  $P = 0.0001$ ) and 3.8-fold in the controls (from  $1.77 \pm 0.19$  to  $6.86 \pm 0.79 \times 10^{-4} \text{ min}^{-1} \cdot \text{per } \mu\text{U/mL}$ ,  $P = 0.0001$ ). However, postpartum insulin sensitivity in the pre-eclamptic group was 26% lower than that in the control group ( $P = 0.04$ ) (Figure 2 and 3). No significant correlation emerged between insulin sensitivity during pregnancy and after delivery in the two groups.

Insulin sensitivity correlated negatively with the weeks of gestation in the pre-eclamptic women ( $r = -0.53$ ,  $P = 0.01$ ), but not in the controls ( $r = -0.13$ ,  $P = 0.64$ ) (Figure 4). Insulin response to oral glucose and insulin sensitivity as measured by the Minimal Model showed a negative correlation in the pre-eclamptic women ( $r = -0.70$ ;  $P = 0.0004$ ), but not in the control women ( $r = -0.45$ ;  $P = 0.10$ ). Furthermore, insulin sensitivity correlated negatively with baseline C-peptide concentrations in both pre-eclamptic women ( $r = -0.62$ ;  $P = 0.002$ ) and control women ( $r = -0.58$ ;  $P = 0.02$ ).



**Fig. 4.** The correlation between insulin sensitivity and weeks of gestation in pre-eclamptic women (●) and normotensive pregnant control women (○).

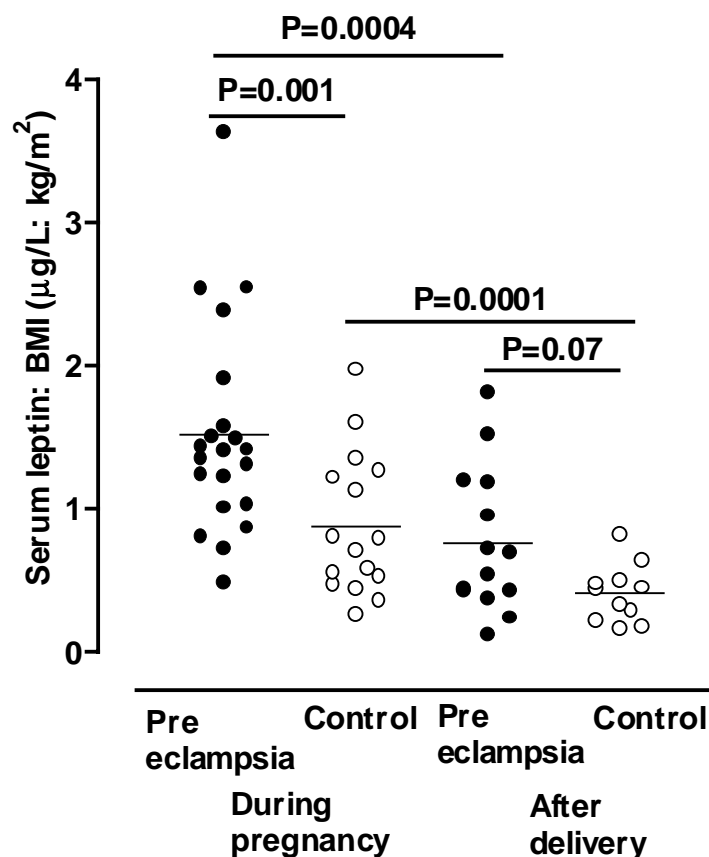
In the pre-eclamptic women, serum triglyceride concentrations were 37% higher ( $P = 0.004$ ) than in the control women ( $3.15 \pm 0.19$  mmol/L vs.  $2.29 \pm 0.21$  mmol/L), but the levels of total and lipoprotein cholesterol did not differ (data not shown). Serum concentrations of HDL<sub>2</sub> cholesterol tended to be lower in pre-eclamptic women ( $0.33 \pm 0.04$  mmol/L) ( $P = 0.09$ ) than in control women ( $0.44 \pm 0.06$  mmol/L). Baseline serum FFA concentrations were 70% higher in preeclamptic women ( $0.17 \pm 0.01$  g/L) than in control women ( $0.10 \pm 0.01$  g/L) ( $P = 0.0004$ ), but this difference disappeared two hours after oral intake of glucose (Figure 5). Oral intake of glucose was accompanied by a 38% reduction FFA in pre-eclamptic women ( $P = 0.0001$ ), but only an 18% reduction in control women ( $P = 0.32$ ) (Figure 5). In the whole study population, insulin sensitivity was negatively related to serum concentrations of triglycerides ( $r = -0.48$ ;  $P = 0.002$ ), but not to those of FFAs or total or lipoprotein cholesterol. In the whole study population the area under the insulin curve during the oral glucose tolerance test was positively related to the reduction in FFAs ( $r = 0.43$ ,  $P = 0.015$ ).



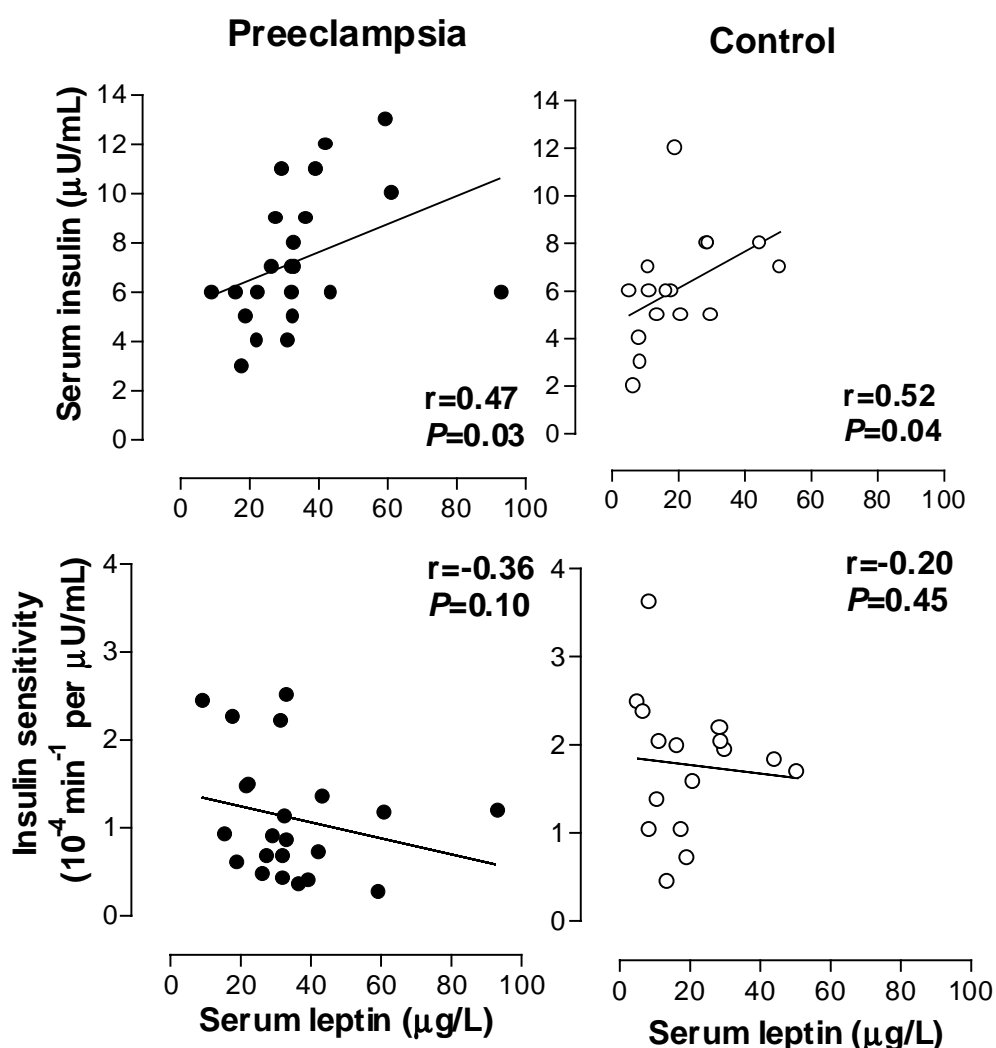
**Fig. 5.** Serum free fatty acids before and following a standard oral glucose dose (75 g) in pre-eclamptic women (●) and normotensive pregnant control women (○). Data are means  $\pm$  SE

## 2. Serum leptin during and after pre-eclamptic pregnancy. Relationship to insulin sensitivity (II)

Leptin levels were significantly higher in pre-eclampsia than in normotensive pregnancy ( $34.6 \pm 3.9$  vs.  $20.0 \pm 3.3$   $\mu\text{g/L}$ ,  $P = 0.002$ ). Also leptin related to BMI in pre-eclamptic women was higher than that in normotensive women (Figure 6). A positive relation between leptin and fasting insulin emerged both during pre-eclampsia ( $r = 0.47$ ,  $P = 0.03$ ) and normal pregnancy ( $r = 0.52$ ,  $P = 0.04$ ) (Figure 7). Insulin sensitivity was not related to leptin levels in either group of women (Figure 7).



**Fig. 6.** Serum leptin levels divided by body mass index (BMI) in 22 pre-eclamptic (●) and 16 control women (○) during pregnancy and at re-examination three months after delivery (14 women with prior preeclampsia and in 11 women with prior normotensive pregnancy). Means indicated by horizontal lines.

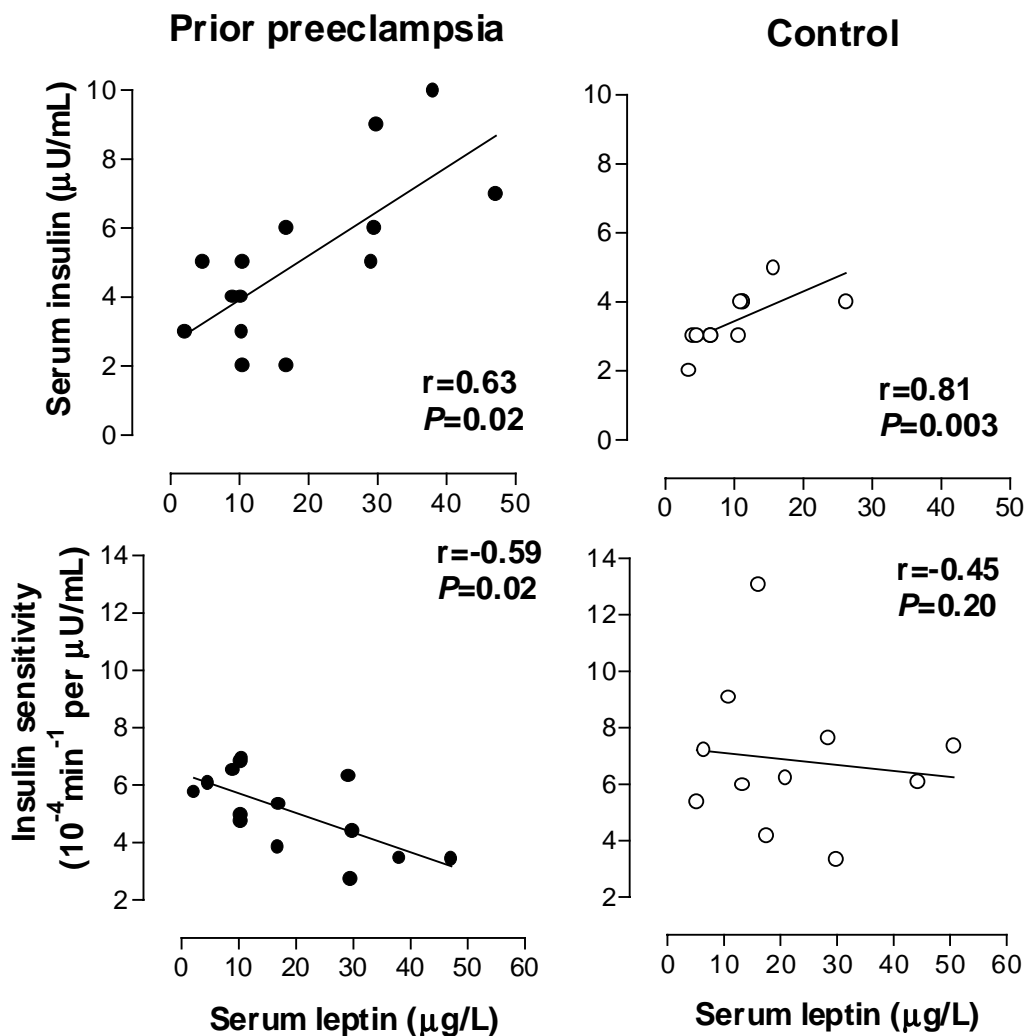


**Fig. 7.** Serum leptin in relation to serum insulin and insulin sensitivity during the third trimester in 22 pre-eclamptic (●) and in 16 control pregnant women (○).

There was a direct correlation between serum leptin concentration and pre-pregnancy BMI in pre-eclamptic women ( $r = 0.49$ ,  $P = 0.02$ ) and in controls ( $r = 0.70$ ,  $P = 0.003$ ), whereas the weight gain during pregnancy was not related to serum leptin concentrations. Serum leptin concentrations correlated with the degree of proteinuria ( $r = 0.46$ ,  $P = 0.03$ ), but not with blood pressure or serum uric acid. Furthermore, no relationships emerged between serum leptin and weight of the infants or of placentas or weeks of gestation in either group.

Serum leptin levels, measured three months after delivery, had decreased significantly in the pre-eclamptic and the control group (Figure 6). Leptin

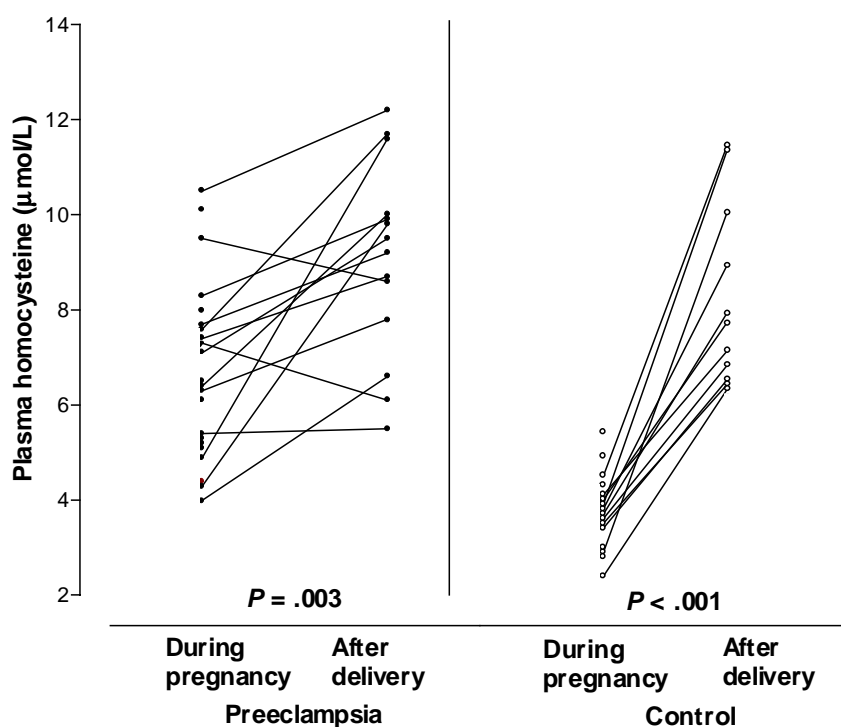
concentrations tended to be higher in puerperal women with prior pre-eclampsia ( $19.0 \pm 3.6 \mu\text{g/L}$ ) than in normal pregnancy ( $10.1 \pm 2.0 \mu\text{g/L}$ ), ( $P = .11$ ). Serum leptin concentrations correlated with those of fasting insulin in the puerperal pre-eclamptic group ( $r = 0.63$ ,  $P = 0.02$ ), and in the control group ( $r = 0.81$ ,  $P = 0.003$ ), and also with insulin sensitivity in the puerperal pre-eclamptic group ( $r = -0.59$ ,  $P = 0.02$ ), but not in the control group (Figure 8). Serum leptin concentrations also correlated with puerperal BMI in the pre-eclamptic ( $r = 0.74$ ,  $P = 0.002$ ) and the control group ( $r = 0.81$ ,  $P = 0.005$ ).



**Fig. 8.** Serum leptin in relation to serum insulin and insulin sensitivity 3 months after delivery in 14 women with prior pre-eclampsia (●) and in 11 women with prior normotensive pregnancy (○).

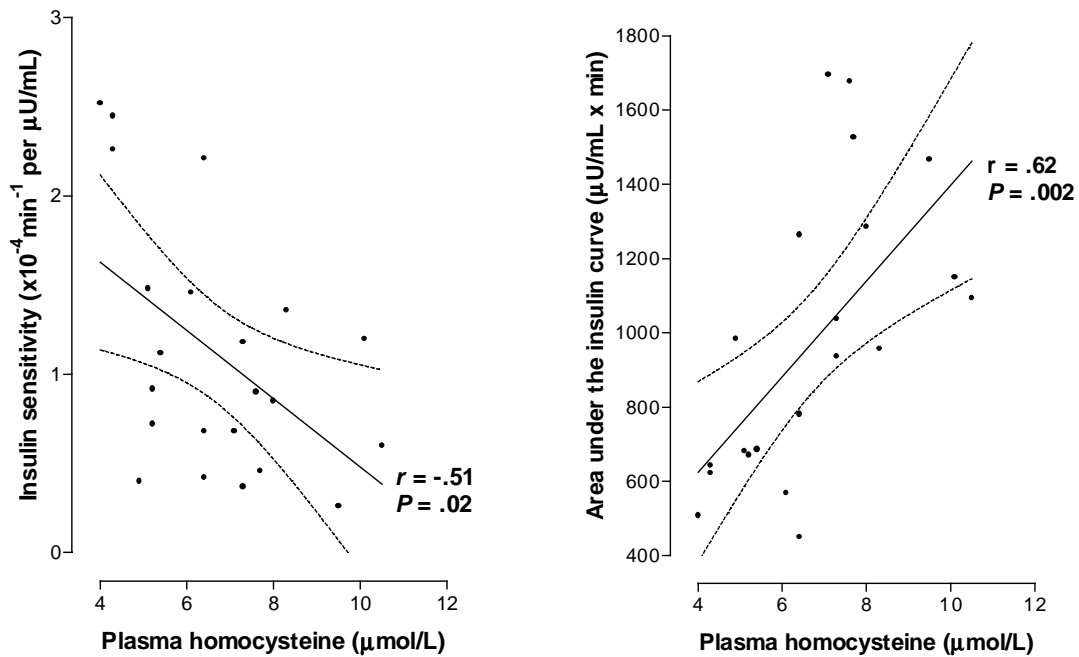
### 3. Plasma homocysteine during and after pre-eclamptic pregnancy. Relationship to insulin sensitivity (III)

Pre-eclamptic women had higher plasma homocysteine concentrations than the controls ( $6.7 \pm 0.4$  vs.  $3.8 \pm 0.2$   $\mu\text{mol/L}$ ) (Figure 9). Three months after delivery, levels of homocysteine (Figure 9) had increased ( $9.1 \pm 0.6$   $\mu\text{mol/L}$  in the women with prior pre-eclampsia, and  $8.2 \pm 0.6$   $\mu\text{mol/L}$  in controls), and showed no difference between the groups.



**Fig. 9.** Fasting homocysteine levels in 22 pre-eclamptic (●) and 16 control (○) women during pregnancy and three months after delivery (14 women with prior pre-eclampsia and 11 women with prior normal pregnancy).

In pre-eclamptic women, plasma homocysteine concentrations showed a significant negative correlation with insulin sensitivity ( $r = -0.51$ ,  $P = 0.02$ ) (Figure 10), and a significant positive correlation with the area under the curve of the first 10-minute insulin levels ( $r = 0.62$ ,  $P = 0.002$ ) during the insulin-sensitivity test (Figure 10). Control women showed no such correlations. The increase in glucose (2.9-fold) and in insulin (52.5-fold) during the insulin sensitivity test were not associated with levels of homocysteine



**Fig. 10.** Plasma homocysteine showed a significant negative correlation to insulin sensitivity (left panel) and a positive correlation to the area under the first ten-minute insulin curve (right panel) in 22 pre-eclamptic women. Dotted lines indicate 95% confidence intervals.

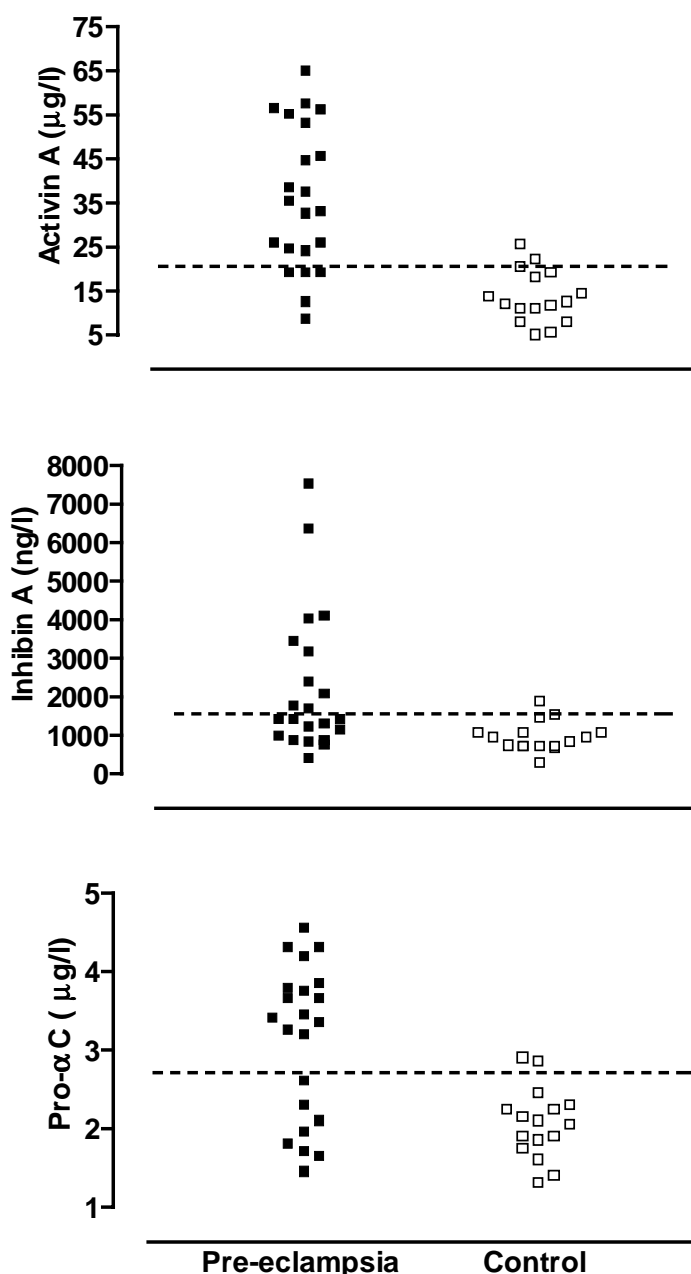
#### 4. Serum activin A, inhibin A and pro- $\alpha$ C inhibin during pre-eclamptic pregnancy. Relationship to insulin sensitivity (IV)

A linear model eliminating the impact of a small difference in gestational age revealed that in pre-eclampsia activin A values were 139% higher, those of inhibin A 39% higher, and those of pro- $\alpha$ C 92% higher than the respective levels in the controls. The weekly increases were 12.5% (95% CI 6.2-19.2%) for activin A, and 4.2% (95% CI 0.1%-8.4%) for pro- $\alpha$ C, whereas no increase was noticed in inhibin A.

Cut-off limits were defined as the 90<sup>th</sup> percentiles of activin A, inhibin A, and pro- $\alpha$ C values in normal pregnancy. The geometric mean and 95% CI of activin A concentrations in pre-eclampsia were 31.8 and 25.0-40.3  $\mu$ g/L, respectively, and in normal pregnancy 12.3 and 9.6-15.9  $\mu$ g/L, respectively ( $P = 0.0001$ ). Seventeen patients (77%) had elevated activin A concentrations (Figure 11). The geometric mean and 95% CI of inhibin A concentrations in pre-eclampsia were 1691 and 1217-

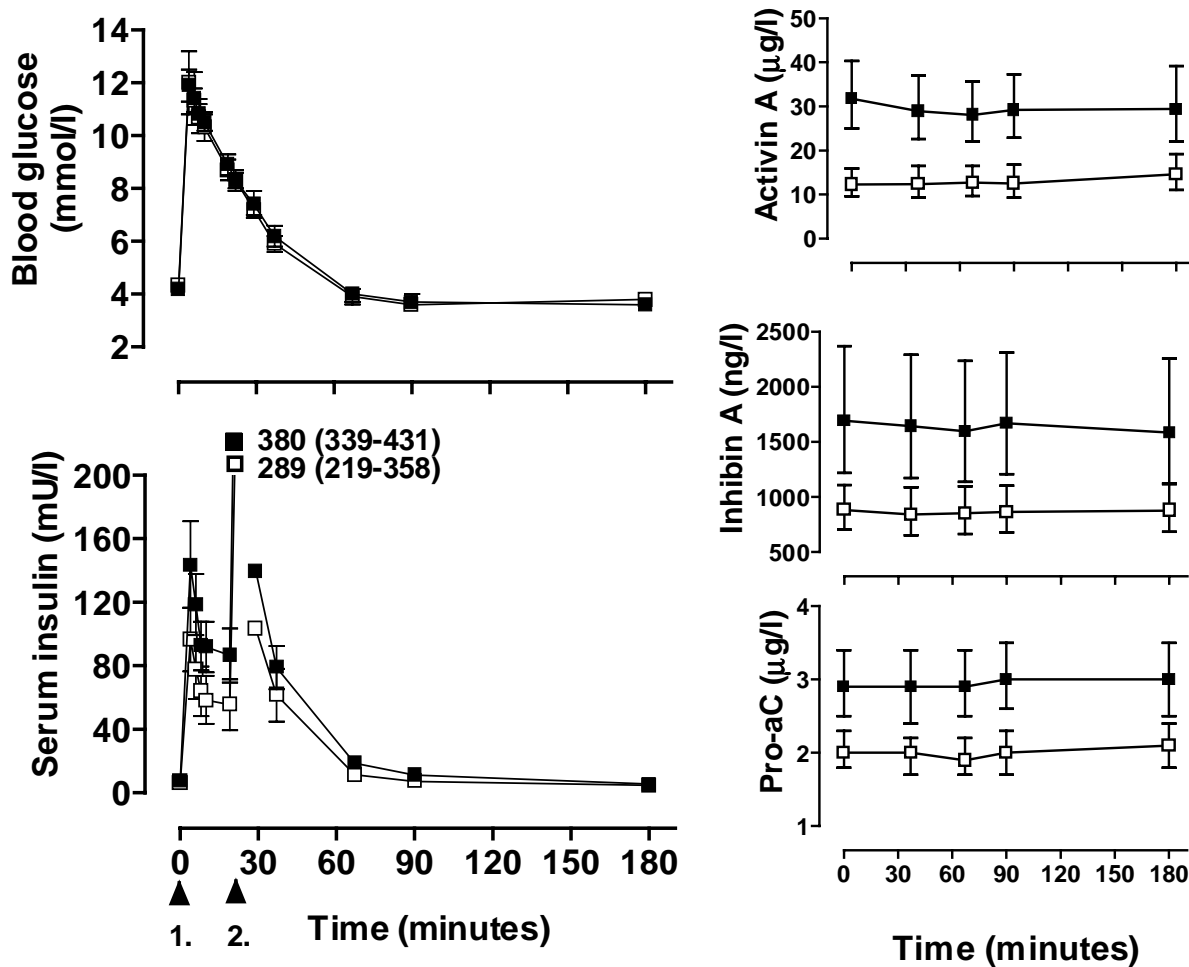


2350 ng/L, respectively, and in normal pregnancy 882 and 703-1107 ng/L, respectively ( $P = 0.003$ ). Ten pre-eclamptic women (46%) had elevated inhibin A concentrations (Figure 11). The geometric mean and 95% CI of pro- $\alpha$ C concentrations in pre-eclampsia were 2.9 and 2.5-3.4  $\mu$ g/L, respectively, and in normal pregnancy 2.0 and 1.8-2.3  $\mu$ g/L, respectively ( $P = 0.0008$ ). Fourteen pre-eclamptic women (64%) had elevated pro- $\alpha$ C concentrations (Figure 11)



**Fig. 11.** Serum levels of activin A, inhibin A, and inhibin pro- $\alpha$ C-containing forms in 22 pre-eclamptic (■) and 16 control (□) women. Dotted lines indicate 90<sup>th</sup> percentiles in the control group.

Activin A, inhibin A and pro- $\alpha$ C concentrations were not related to insulin sensitivity in either group. The mean increases in blood glucose (2.9-fold) and in serum insulin (52.5-fold) during Minimal Model testing were not associated with any changes in activin A, inhibin A, or pro- $\alpha$ C concentrations, which however, were consistently higher in pre-eclampsia (Figure 12).



**Fig. 12.** Levels of activin A, inhibin A and pro- $\alpha$ C inhibin (geometric mean and 95% CI), blood glucose, and serum insulin (arithmetic mean and 95% CI) before and during Minimal Model testing in 22 pre-eclamptic (■) and 16 control (□) women. 1.= Glucose at 0.3 g/kg i.v. (time 0), 2.= Insulin at 0.03 IU/kg i.v. (20 min).

## 5. Insulin sensitivity, lipids and lipoproteins during isradipine and metoprolol treatment in pre-eclamptic pregnancy (V)

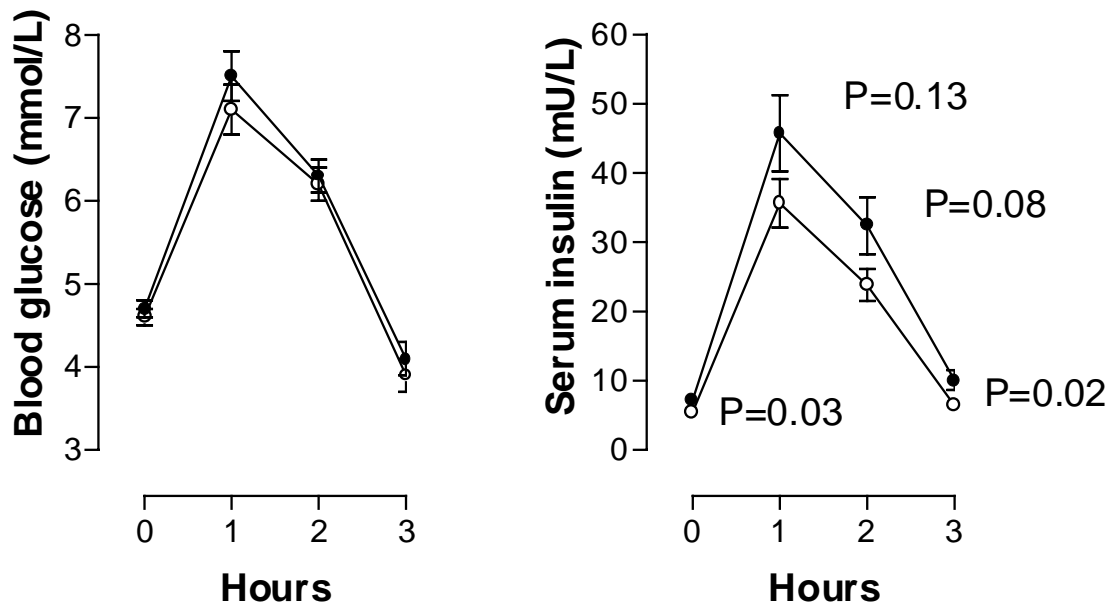
After 5 to 7 days of isradipine treatment, fasting glucose concentrations tended to decrease [-0.12 (-0.27-0.03) mmol/L] ( $P = 0.09$ ), whereas no such effect was seen in the metoprolol group [0.16 (-0.10-0.43) mmol/L] ( $P = 0.19$ ). The difference in fasting glucose at day 5-7 was significant between the two groups ( $P < 0.05$ ). Isradipine and metoprolol had no effect on insulin sensitivity, although the glucose response during the first 10 minutes in the intravenous glucose tolerance test tended to decrease in the isradipine group ( $P = 0.10$ ).

High-density lipoprotein<sub>2</sub> (HDL<sub>2</sub>) cholesterol concentrations increased in the isradipine group by 0.07 (0.0008-0.14) mmol/L ( $P < 0.05$ ), but remained unchanged in the metoprolol group ( $P = 0.81$ ). The difference in HDL<sub>2</sub> values between the two groups was significant at reinvestigation: isradipine group 0.52 (0.34-0.70) mmol/L vs. metoprolol group 0.32 (0.19-0.44) mmol/L ( $P < 0.05$ ). No significant changes occurred among other lipids and lipoproteins in the two groups before or during antihypertensive treatment.

Isradipine and metoprolol caused a non-significant reduction in BP. A non-significant decrease in the isradipine group [from 0.66 (0.60-0.71) to 0.62 (0.54-0.73),  $P = 0.48$ ] and increase in the metoprolol group [from 0.63 (0.57-0.68) to 0.66 (0.61-0.71),  $P = 0.14$ ] occurred in the umbilical artery resistance index.

## 6. Glucose tolerance, lipids and lipoproteins in women with prior pre-eclamptic pregnancy (VI)

Blood glucose in the two groups at baseline and during the OGTT were comparable (Figure 13). In the pre-eclamptic group two women showed elevated 2-hour glucose levels (8.6 mmol/l and 7.8 mmol/L, respectively). Serum insulin levels in the pre-eclamptic group were elevated at baseline ( $7.3 \pm 0.6$  vs.  $5.5 \pm 0.5$  mU/L,  $P = 0.03$ ), at 1 hour ( $45.7 \pm 5.5$  vs.  $35.6 \pm 3.5$  mU/L,  $P = 0.13$ ), at 2 hours ( $32.4 \pm 4.1$  vs.  $23.8 \pm 2.3$  mU/L,  $P = 0.08$ ), and at 3 hours ( $10.1 \pm 1.4$  vs.  $6.4 \pm 0.6$  mU/L,  $P = 0.02$ ) (Figure 13).



**Fig. 13.** Blood glucose and serum insulin (mean  $\pm$  SE) before and following oral intake of 75 g glucose in women with a history of pre-eclampsia or eclampsia (●) and in control women (○).

The AUC for insulin was larger in the pre-eclamptic/eclamptic group than in the control group ( $86.8 \pm 9.1$  vs.  $65.4 \pm 5.2$  mU/L  $\times$  h,  $P = 0.05$ ) whereas the AUC for glucose was similar in the two groups ( $18.2 \pm 0.6$   $\times$  hr vs.  $17.6 \pm 0.4$  mmol/L  $\times$  h). The elevated basal serum insulin levels in the pre-eclamptic/eclamptic group remained so even when insulin was adjusted for BMI.

Total cholesterol, LDL and HDL cholesterol, triglyceride and uric acid concentrations did not differ significantly between the two groups.

In the pre-eclamptic/eclamptic group, fasting serum insulin correlated positively with serum triglyceride ( $r = 0.59$ ;  $P < 0.01$ ), BMI ( $r = 0.54$ ;  $P < 0.01$ ) and systolic ( $r = 0.69$ ;  $P < 0.001$ ) and diastolic ( $r = 0.47$ ;  $P < 0.05$ ) blood pressure. All these variables remained significant even when analysed in the multiple regression model. The AUC for insulin in the pre-eclamptic/eclamptic group correlated positively with serum triglycerides ( $r = 0.57$ ;  $P < 0.01$ ), BMI ( $r = 0.49$ ;  $P < 0.05$ ) and systolic blood pressure ( $r = 0.53$ ;  $P < 0.05$ ) and negatively with HDL<sub>2</sub>-cholesterol ( $r = -0.48$ ;  $P < 0.05$ ). The AUC for insulin did not differ between those who developed pre-eclampsia or hypertension in subsequent pregnancies and those who did not.

## 7. Sex hormones and endothelial markers in women with prior pre-eclamptic pregnancy (VII)

Women with prior pre-eclampsia were characterized by elevated serum free testosterone levels ( $20.6 \pm 2.2$  vs.  $15.0 \pm 0.3$  pmol/L,  $P = 0.03$ ), “free androgen index“  $3.2 \pm 0.5$  vs.  $1.9 \pm 0.2$ ,  $P = 0.04$ ) and free testosterone / estradiol ratio ( $0.089 \pm 0.017$  vs  $0.046 \pm 0.006$ ,  $P = 0.02$ ). Serum concentrations of androstenedione, dehydroepiandrosterone sulphate, oestradiol and total testosterone were normal. Free testosterone correlated positively with basal insulin ( $r = 0.52$ ,  $P = 0.016$ ), systolic blood pressure ( $r = 0.69$ ,  $P = 0.001$ ), diastolic blood pressure ( $r = 0.62$ ,  $P = 0.004$ ), triglycerides ( $r = 0.55$ ,  $P = 0.009$ ) and BMI ( $r = 0.51$ ,  $P = 0.018$ ) in women with prior pre-eclampsia, but not in the controls. The study groups did not differ with respect to SHBG, thyroxine, LH, or FSH concentrations or the LH/FSH ratio.

Plasma endothelin-1 concentrations as well as the urinary output of PGI<sub>2</sub> and TXA<sub>2</sub> metabolites were similar in the two groups, and showed no correlation with steroid hormone concentrations.

The oral glucose tolerance test resulted in a progressive fall in insulin-like growth factor binding protein-1 (IGFBP-1) levels, but this response, as well as the basal levels of IGFBP-1, did not differ between the study groups. Basal serum IGFBP-1 and insulin concentrations were negatively correlated to each other ( $r = -0.49$ ,  $P = 0.001$ ).

## VI DISCUSSION

Insulin resistance has attracted much interest in internal medicine, because it leads to the development of CVD in otherwise healthy subjects (Reaven 1994). Our studies were primarily focused on evaluation of whether pre-eclampsia is characterized by similar metabolic changes as are known to occur in insulin resistance syndrome. We included only nulliparous pre-eclamptic women who had been healthy before the 20th gestational week, and whose pre-eclamptic signs had disappeared six weeks postpartum. Thus we are convinced that our patients had clear-cut pure pre-eclampsia (Gifford *et al.* 1990). In addition, we studied insulin and insulin sensitivity to see if they may be factors in some other variables which have been linked to insulin resistance or vascular disorders outside pregnancy. Finally, we

also studied metabolic and endocrine changes 17 years after pre-eclamptic pregnancy in order to see if insulin resistance and other signs possibly associated with it prevail in women with a history of pre-eclampsia.

To assess insulin sensitivity we used the Minimal Model technique, which is a representative and reliable method for this purpose (Bergman 1989). The Minimal Model has the advantage of measuring insulin sensitivity in terms of fractional glucose disappearance rates, which should remain independent of the changes in body composition during pregnancy (Buchanan 1997). An additional obstacle to the study of insulin sensitivity in pregnancy is the decrease in insulin sensitivity over the course of gestation (Cousins 1991). It has been reported that Minimal Model analysis may yield negative values for insulin sensitivity in severe insulin resistance (Saad *et al.* 1994), but negative values in the Minimal Model did not appear in this study. The hyperinsulinaemic euglycaemic clamp shows better reproducibility in non-pregnant individuals than do other methods (coefficient of variation 10%) (DeFronzo *et al.* 1979), but its use in pregnant women is not without problems. Changes in body composition and weight complicate the measurement of insulin sensitivity by means of the hyperinsulinaemic euglycaemic clamp method during pregnancy. Although correlation between insulin sensitivities assessed by the Minimal Model and hyperinsulinaemic euglycaemic clamp-derived values may be poorer in late pregnancy than in non-pregnant women, both methods give comparable results showing good correlation ( $r = 0.58$ ) in pregnancy (Buchanan 1997).

Insulin sensitivity in pre-eclampsia was 37% lower than that in normotensive pregnancy. This new finding is in line with previous data on elevated fasting insulin (Kaaja *et al.* 1995, Martinez Abundis *et al.* 1996) and post-load insulin levels (Martinez Abundis *et al.* 1996, Lorentzen *et al.* 1998) in pre-eclampsia. It is also known that the incidence of pre-eclampsia is increased in insulin-resistant states such as GDM (Suhonen and Teramo 1993) and obesity (Ros *et al.* 1998) which may suggest that decreased insulin sensitivity precedes the onset of pre-eclampsia. However, the data on decreased insulin sensitivity in pre-eclampsia are not uniform, since both normal (Caruso *et al.* 1999) and increased (Roberts *et al.* 1998) insulin sensitivity have been reported. Different methods, different populations, and heterogeneous origin of pre-eclampsia (placental and maternal factors) may be explanations for the discrepant results.

The cause of decreased insulin sensitivity in normal pregnancy (Cousins 1991) is unknown, but several pregnancy-associated hormones, such as human placental lactogen, cortisol, oestrogens, progesterone and prolactin may be involved (Ryan and Enns 1988, Murai *et al.* 1997). Elevated levels of FFAs may also contribute to the decreased insulin sensitivity (Sivan *et al.* 1998), as does sympathetic over-activity (Schobel *et al.* 1996, Manyonda *et al.* 1998), which characterises pre-eclampsia. These physiological responses to pregnancy may be exaggerated or otherwise altered in pre-eclampsia so that they may become responsible for the decreased insulin sensitivity in these women. One hypothesis explains decreased insulin sensitivity in pre-eclampsia through increased FFA levels and lipid oxidation, which together with increased cytokine levels may enhance insulin-independent glucose transport via the hexosamine pathway. In obesity, elevated serum free fatty acid concentrations and enhanced lipid oxidation (Groop *et al.* 1992) induce skeletal muscle insulin resistance by increasing the flux of glucose into the hexosamine pathway (Hawkins *et al.* 1997), and obesity is a known risk factor as regards pre-eclampsia. Hexosamines inhibit the insulin-dependent glucose transport protein (GLUT) 4, the predominant glucose transporter in anabolic conditions (Ebeling *et al.* 1998). If glucose enters the cell via the insulin-independent GLUT 1 and activates hexosamine pathway, this hypothesis can also explain why insulin resistance may be associated with fasting hypoglycaemia, as was the tendency in present study. Insulin-dependent glucose transport may be already impaired in women predisposed to pre-eclampsia. Impaired glucose phosphorylation, catalyzed in muscle by hexokinase (HK) II, has been suggested to contribute to insulin resistance in obesity and NIDDM (Pendergrass *et al.* 1998).

Decreased insulin sensitivity may impair endothelial cell function by decreasing PGI<sub>2</sub> production (Axelrod 1991), or by increasing ET-1 production (Ferri *et al.* 1996). Another factor could be the effect of insulin resistance on lipids and lipoproteins, such as increased VLDL secretion, affecting endothelial cell function and favouring vasoconstriction (Sattar *et al.* 1996). It is not known whether insulin resistance in pre-eclampsia is a cause or a consequence of endothelial dysfunction. Substances such as nitric oxide donors and antioxidants may be useful tools to study these relationships.

Our finding of decreased insulin sensitivity in the postpartum period in Finnish pre-eclamptic women is in agreement with some previous data on Chinese women (Fuh

*et al.* 1995), obtained two months postpartum by means of the insulin suppression test. However, our results are in disagreement with data on African-American women (Jacobson *et al.* 1994) obtained 3-6 months postpartum with the hyperinsulinaemic-euglycaemic clamp technique. These discrepancies might be explained by differences in methods of measuring insulin sensitivity, or in intervals between delivery and each study. A difference in race can also be a confounding factor.

Our data show that leptin concentrations are elevated in pre-eclampsia. Leptin is eliminated mainly through the kidneys (Stenvinkel *et al.* 1997, Merabet *et al.* 1997), and pre-eclampsia can be accompanied by reduced renal blood flow and a reduced glomerular filtration rate (August 1993). The correlation between serum leptin and proteinuria in our patients suggests an association, either direct or indirect, between the elevation in serum leptin levels and renal changes in pre-eclampsia, although it must be understood that proteinuria in pre-eclampsia is not a sign of renal insufficiency, and in effect, none of our patients had renal insufficiency. It is also possible that elevated leptin levels in pre-eclampsia are due to release from placental sources (Masuzaki *et al.* 1997, Mise *et al.* 1998) perhaps as a reaction to placental hypoxaemia (Mise *et al.* 1998). However, in our study leptin levels were not associated with the birth weight, placental weight or Apgar scores.

It was a curious and new finding that leptin levels remained elevated in pre-eclamptic women up to three months after delivery. Our data are the first to show that insulin sensitivity is not associated with leptin levels in pregnancy. However, such an association exists in non-pregnant subjects (Segal *et al.* 1996, Haffner *et al.* 1997). It is possible that during pregnancy placental leptin production may have masked an association between insulin sensitivity and leptin, and this gains support from an association with insulin sensitivity in puerperal women who had had pre-eclampsia. Taken as a whole, our data suggest that hyperleptinaemia might be listed among the metabolic alterations characteristic of women with prior pre-eclampsia.

Plasma homocysteine levels in pre-eclamptic patients were on average 1.8 times higher than those in control women. This finding in a Finnish population appears to be in agreement with data from the USA (Rajkovic *et al.* 1997, Powers *et al.* 1998). However, the demonstration of a relationship between homocysteine levels and severity of pre-eclampsia, and an inverse correlation between plasma homocysteine and insulin sensitivity are new findings. We do not know whether or not this is a



cause and effect relationship reflecting perhaps some common factor in vascular physiology. Probably this connection is based on long-term effects, since at dramatic short-term changes in circulating glucose and insulin during Minimal Model failed to affect homocysteine levels.

Maternally derived homocysteine is transported by the placenta into the umbilical vein, where it is extracted by the foetus (Malinow *et al.* 1998). This could explain the inverse correlation between infant birth weight and maternal plasma homocysteine in our study; reformation of methionine from homocysteine for foetal demands may have been less in pre-eclamptic women with smaller foetuses. A relative reduction in vitamin B<sub>12</sub> concentrations in pre-eclampsia, as seen in our patients, may contribute to this effect, because vitamin B<sub>12</sub> is essential for the reformation of methionine from homocysteine (Welch and Loscalzo 1998). It is also possible that a common mutation in the methylenetetrahydrofolate reductase gene associated with higher homocysteine levels (Sohda *et al.* 1997) could have been more frequent pre-eclamptic women than controls. The frequency of homozygosity of this mutation is in the order of 5-10% in different European populations (Gudnason *et al.* 1998). It appears unlikely, however, that many of our pre-eclamptic patients would have had this mutation, since no difference emerged in plasma homocysteine values between the groups at postpartum examination. This does not exclude the possibility that these women could have had a genetic factor or some other tendency leading towards elevated levels of homocysteine, something which perhaps can be determined only with the aid of a methionine-loading test (Dekker *et al.* 1995).

Serum concentrations of activin A, inhibin A and pro- $\alpha$ C are markedly elevated in pre-eclampsia, and we found these elevations to be related to the amount of proteinuria. Our data failed to show any conclusive relationship between insulin sensitivity and activin A, inhibin A, or pro- $\alpha$ C in pre-eclampsia. Moreover, acute, and dramatic rises in insulin and glucose during the testing of insulin sensitivity did not affect the circulating levels of these glycoproteins during the subsequent 160-180 minutes, either in pre-eclampsia or in normal pregnancy. In contrast to the results of one previous study (Muttukrishna *et al.* 1997), we observed significant overlapping of the levels of activin A and inhibin A between pre-eclamptic and normotensive pregnancies, a fact which may decrease the value of these tests in the diagnosis of pre-eclampsia. Differences in stage of pre-eclampsia or in genetic background

affecting both pre-eclampsia and these proteins, or small sample sizes, may explain these discrepancies.

Metoprolol given for more than 3 months is associated with a 14 to 22% decrease in insulin sensitivity in men and non-pregnant women with essential hypertension (Haenni and Lithell 1994, Jacob *et al.* 1996), whereas isradipine, on the other hand, has been considered to be neutral in this regard (Brogden and Sorkin 1995). Our data show that short-term isradipine and metoprolol treatment had no detrimental effects on insulin sensitivity or serum lipid and lipoprotein levels in pre-eclamptic pregnancies. Sympathetic over-activity (Schobel *et al.* 1996) and pre-existing insulin resistance, as shown in our study, may have prevented any possible adverse effect of metoprolol on insulin sensitivity in pre-eclampsia. Our patients were insulin resistant, and it has been suggested that the  $\beta$ -blocker-induced reduction in insulin sensitivity may be greater in patients with higher initial insulin sensitivity (Malminiemi *et al.* 1997). It is also possible that both isradipine and metoprolol may have prevented the possible worsening of insulin resistance, along with the progression of pre-eclampsia, as seen by a rise in uric acid, but to exclude this possibility we would have had to have had a control group of pre-eclamptic patients followed without antihypertensive treatment. For ethical reasons this was not possible.

Increases in serum triglycerides and decreases in HDL cholesterol have been reported during  $\beta$ -blocker treatment in non-pregnant women (Ames 1986), but this does not occur in pre-eclamptic patients, as observed in our study. In contrast, HDL<sub>2</sub> cholesterol concentrations increased during the short-term treatment with isradipine. This effect of isradipine may be beneficial for endothelial cell function, and, for example, it may stimulate vasodilatory PGI<sub>2</sub>-release from vascular endothelium (Pomerantz *et al.* 1985, Kaaja *et al.* 1995, Tamagaki *et al.* 1996).

The concentrations of FFAs in serum were elevated in pre-clampsia in the present study. The cause of this change cannot be explained by the present data, but it may be possible that this is one sign of insulin resistance on the adipocytes. It was, however, curious that the levels of FFA reduced markedly during OGTT. This could perhaps be seen as a piece of evidence that hormone sensitive lipase could be suppressed by high levels of insulin following glucose challenge.

Women with a history of pre-eclampsia, studied 17 years after their first pregnancy, were characterized by elevated fasting insulin levels, an exaggerated insulin response in the OGTT, and elevated systolic and diastolic blood pressures.

These findings suggest that women who once had had pre-eclampsia, may have insulin resistance. In the pre-eclamptic group the magnitude of hyperinsulinaemia (and thus perhaps insulin resistance) correlated negatively with HDL cholesterol, but positively with triglyceride concentrations and with systolic and diastolic blood pressure. Lipid and lipoprotein concentrations did not differ significantly. It is possible that the ability of insulin to stimulate the uptake and disposal of glucose by peripheral tissues precedes the changes in lipid and lipoprotein levels.

We found mild hyperandrogenism, reflected in increased free testosterone, an increased free androgen index and an increased free testosterone/oestradiol ratio in women with prior pre-eclampsia. These changes could result from increased ovarian testosterone production or decreased circulating SHBG levels, or both, but our data do not allow us to deduce the initial cause of these changes. Regardless of the cause of testosterone changes, and of the presence of normal androstenedione levels, we feel that our patients had slight ovarian hyperandrogenism. We do not know which of the two major abnormalities, hyperinsulinaemia or high levels of testosterone, is the primary change. Insulin stimulates the production of testosterone by ovarian tissue *in vitro* (Barbieri *et al.* 1986), which suggests that hyperinsulinaemia could be the primary change which triggers increased release of testosterone. On the other hand, androgens are known to decrease both hepatic removal of insulin and peripheral sensitivity to insulin (Peiris *et al.* 1987), which suggests that hyperandrogenism could lead to hyperinsulinaemia. Similar coexistence of hyperinsulinaemia and hyperandrogenism is present in PCOS and these patients appear to be at an increased risk of pre-eclampsia (Diamant *et al.* 1982). This suggests that hyperandrogenism could precede the onset of pre-eclampsia. Moreover, it has been reported that administration of phosphoglycan, which increases the action of insulin, improves ovulatory function, and decreases serum androgen concentrations, blood pressure, and plasma triglycerides in women with PCOS (Nestler *et al.* 1999).

Prostacyclin and TxA<sub>2</sub> are important in pregnancy physiology and in pre-eclampsia (Ylikorkala and Viinikka 1992) in which ET-1 production can also be elevated (Taylor *et al.* 1990). Our data are the first demonstration that PGI<sub>2</sub> deficiency and/or TxA<sub>2</sub> or ET-1 dominance, which characterize pre-eclamptic pregnancy (Ylikorkala and Viinikka 1992) have totally vanished within 17 years after pregnancy. Thus, these

vascular factors are not likely to account for the increased risk of CVD in these women (Jónsdóttir *et al.* 1995, Hannaford *et al.* 1997).

## VII SUMMARY AND CONCLUSIONS

We studied whether the metabolic characteristics of so-called insulin resistance syndrome are associated with acute pre-eclampsia and prevail after pre-eclamptic pregnancy. To this end, insulin sensitivity was measured by the intravenous glucose tolerance test (the Minimal Model method) in 31 women with pre-eclampsia and 16 women with normotensive pregnancy. In pre-eclampsia, insulin sensitivity is reduced on average by 37%, whereas serum FFA and triglyceride concentrations are elevated. Insulin sensitivity was reduced by 26% three months after delivery. Serum leptin, also possibly a part of the insulin resistance syndrome, was 73% higher in pre-eclamptic women than in control women, and correlated with the level of proteinuria. Leptin concentrations were related to those of insulin both in pre-eclampsia and normal pregnancy, but not to insulin sensitivity. Serum concentrations of leptin remained elevated in puerperal women with prior pre-eclampsia, and only in these women did they show a relationship to insulin sensitivity. Concentrations of plasma homocysteine, a detrimental vascular factor outside pregnancy, was 76% higher in pre-eclamptic women, and they showed a significant relationship to the level of proteinuria. Homocysteine levels were negatively related to insulin sensitivity in pre-eclamptic women, but unaffected by dramatic changes in glucose and insulin levels during Minimal Model testing. Activin A, inhibin A and pro- $\alpha$ C are placentally derived glycoproteins which were elevated in pre-eclampsia, but in no relationship to insulin sensitivity. The calcium channel blocker isradipine and the  $\beta$ -blocker metoprolol did not affect insulin sensitivity, but isradipine increased HDL<sub>2</sub> cholesterol concentrations in pre-eclamptic women.

Twenty-two women with prior pre-eclampsia (17 years earlier) and 22 control women were studied by way of a standardized oral glucose tolerance test. Fasting insulin levels were elevated by 33%, 3-hour insulin levels were elevated by 58%, and the area under the insulin curve was increased by 33% in women with prior pre-eclampsia. Lipid and lipoprotein concentrations were normal. In addition, these women had elevated serum free testosterone levels (37%), an elevated free androgen index (68%), and an elevated free testosterone to oestradiol ratio (93%).

The levels of insulin-like growth factor-binding protein-1, FSH, LH, androstenedione, dehydroepiandrosterone sulphate and ET-1 as well as urinary output of PGI<sub>2</sub> and TxA<sub>2</sub> metabolites were normal in these women.

Our studies show evidence that pre-eclampsia is a state of increased insulin resistance, which persists for at least three months after pregnancy but may do so for up to 17 years. High leptin and, homocysteine levels may be reflections, at least in part, of altered glucose homeostasis in pre-eclampsia. Isradipine and metoprolol have no detrimental effect on insulin sensitivity and can be safely used, from this point of view, in pre-eclampsia. The data of the present study do not allow to deduce whether insulin resistance in pre-eclamptic women is a primary change, or whether it is secondary e.g. to endothelial dysfunction. Increased insulin levels after pre-eclamptic pregnancy, as seen in present study, may suggest that the insulin resistance is an inherent property in women with pre-eclampsia. The data imply that insulin resistance may perhaps be an aetiopathogenetic factor in pre-eclampsia, prevailing long after pregnancy, and perhaps explaining the increased incidence of cardiovascular morbidity in these women.

## ACKNOWLEDGEMENTS

The present study was carried out at the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, during the years 1994-1998. I wish to express my gratitude to professor *Olavi Ylikorkala*, M.D., Ph.D., and professor *Markku Seppälä*, M.D., Ph.D., Heads of the Departments of Obstetrics and Gynaecology, for placing the excellent facilities at my disposal.

I would like to express my thanks to all the following:

Above all, professor *Olavi Ylikorkala*, M.D., Ph.D., my teacher and supervisor, who has provided a starting point in my scientific research. He has offered constant advice and encouragement through every phase of this study's preparation and unstinting support in perusing the countless pages.

Docent *Risto Kaaja*, M.D., Ph.D., my supervisor, for various advice, guidance and answers to queries. To him my gratitude is also far ranging.

Professor *James Roberts*, M.D., Ph.D. and professor *Jaakko Tuomilehto*, M.D., Ph.D. No usual thanks are sufficient to acknowledge my debt to these generous colleagues who read drafts of my book and improved it with their comments.

Professor *Markku Seppälä*, M.D., Ph.D. for inspiring words and geniality.

Professor *Matti Tikkanen*, M.D., Ph.D. and professor *Markku Laakso*, M.D., Ph.D., who were generous in offering the assistance of their own wide knowledge and experience in the field of medicine.

Docent *Eeva-Marja Rutanen*, M.D., Ph.D., who has set an example of combining clinical and scientific work, for her collaboration and sympathetic interest and docent *Bruno Cacciatore*, M.D., Ph.D., for his expertise in ultrasound, docent *Ulf-Håkan Stenman*, M.D., Ph.D., *Ursula Turpeinen*, Ph.D., and docent *Lasse Viinikka*, M.D., Ph.D., the fine scientists with whom I had a good fortune to collaborate.

Docent *Sture Andersson* M.D., Ph.D, *Sirkka-Liisa Karonen*, Ph.D., *Heikki Koistinen*, M.D., Ph.D, and professor *Veikko Koivisto*, M.D., Ph.D, for pleasant collaboration, and for offering innumerable helpful and thought-provoking comments.

Ms. *Laila Selkinen*, for irreplaceable help in all possible situations, at all possible times.

Ms. *Leena Järvinen*, Ms. *Marja-Leena Pekonen* and Ms. *Anna-Liisa Vuohijoki* for their excellent assistance.

The personnel of the prenatal clinic, the antenatal ward and the delivery ward for help and co-operation, and the staff of the laboratory of the prenatal clinics for pleasant atmosphere in everyday work.

The volunteers for making this study possible, and for their unselfish and positive attitude towards research.

*Nicholas Bolton*, Ph.D., for skilful revising of the language.

Professor *Outi Hovatta*, M.D., Ph.D, docent *Pauli Kajanoja*, M.D., Ph.D., docent *Helena Kääriäinen*, M.D., Ph.D, docent *Riitta Salonen*, M.D., Ph.D., and my other colleagues who have helped and taught me, when I was shuttling between ob & gyn and clinical genetics.

The Academy of Finland, the Clinical Research Institute and the research funds of the Helsinki University Central Hospital, and the Obstetrics and Gynecological Research Foundation for financial support. Novartis Finland Oy and Leiras Oy for providing the medication used in Study V

All my family, thank you for everything!

Espoo, September 1999

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