Uterine artery Doppler velocimetry, calcium and lipids in pregnant women of advanced age

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Uterine artery Doppler velocimetry, calcium and lipids in pregnant women of advanced age

Doppler bloedstroomsnelheidsprofielen van de arteria uterina, calcium en lipiden in oudere zwangere vrouwen

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PUBLICATIONS BASED ON THE STUDIES DESCRIBED IN THIS THESIS

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Elzen HJ van den, Cohen-Overbeek TE, Grobbee DE, Wladimiroff JW. The predictive value of uterine artery flow velocity waveforms in spontaneous abortion in older women. Br J Obstet Gynaecol 1992, in press.

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Chapter 4.1
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Chapter 4.3
Elzen HJ van den, Wladimiroff JW, Cohen-Overbeek TE, Bruijn AJ de, Grobbee DE. Serum lipids in early pregnancy and the risk of preeclampsia (submitted).

CHAPTER 1

INTRODUCTION AND DEFINTION OF OBJECTIVES

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INTRODUCTION AND DEFINITION OF OBJECTIVES

In recent years, in westernised societies a trend has become apparent of an increasing birth rate in women of advanced maternal age. The shift in age of first childbearing towards the mid-thirties results not only from a growing number of women in the age group of 35-45 years, but also reflects an increased fertility of these women[1]. The pursuit of educational goals and professional career before starting a family, late and second mariages, financial concerns and control over fertility and infertility are considered to have contributed to the delay of childbearing[2,3,4]. Women of advanced maternal age have long been considered to be at increased risk of both fetal and maternal morbidity[5], although recent studies on this subject have provided conflicting or inconsistent results[6,7] with the rates of preterm delivery, small-for-gestational-age infants and perinatal morbidity and mortality being both higher or similar to those in younger women. Little doubt, however, exists regarding a decreased ability to conceive[8] in older women and a risk of spontaneous abortion, which increases steeply after the age of 35[9]. In addition, advanced maternal age is associated with increased rates of hypertensive disorders of pregnancy and diabetes[7].

Although a full understanding of the pathophysiological basis explaining the increased risk of pregnancy complications with advancing maternal age is still lacking, an important role has generally been assigned to a decreased uteroplacental circulation[6]. The possibility that age might induce alterations in uterine blood flow was first suggested by Naeye[10], who observed a progressive development of sclerotic lesions of the intramyometrial arteries with advancing age. During pregnancy these lesions might restrict the luminal expansion of the myometrial arteries, resulting in a restricted blood flow to the placenta. In normal pregnancy the spiral arteries in the placental bed are converted into uteroplacental arteries through throphoblast invasion in the walls of these vessels[11]. These vascular adaptations have two closely related effects on the uteroplacental circulation. Due to loss of the musculo-elastic tissue the uteroplacental arteries are no longer responsive to circulating pressor agents and vascular resistance of the placental bed decreases resulting in an increased flow rate. Absent or inadequate vascular changes have been observed in pregnancies complicated by hypertensive disorders and intrauterine growth retardation[12], as well as in women suffering from recurrent spontaneous abortion[13]. Possibly, the sclerotic lesions observed by Naeye[10] restrict the normal histologic response to pregnancy and predispose older women to pathological conditions associated with deficient throphoblast invasion.

Doppler ultrasound examination of the uteroplacental arteries allows non-invasive exploration of uteroplacental hemodynamics[14]. Spectral analysis of the blood flow velocity waveform, together with ratios used to describe the velocity signal provides qualitative information regarding blood velocities in the vessel under study. One of the blood flow velocity waveform characteristics is blood flow pulsatility, which reflects the degree to which the pressure induced flow velocity is tempered by peripheral vascular resistance[15]. The pulsatility index (PI), defined as the maximum systolic frequency minus the minimum diastolic frequency divided by the mean over the cardiac cycle[15], is used in the studies presented in this thesis to describe the uterine artery blood flow velocity waveform and, hence, provide indirect information on uteroplacental vascular resistance.
Uterine artery PI was studied longitudinally in a cohort of 393 pregnant women of 35 years and older. The following primary research questions were addressed:

1) Do flow velocity waveforms from the uterine artery change during the first and second trimester of pregnancy? If so, are these changes associated with maternal age?

2) Are flow velocity waveforms from the uterine artery in the first and second trimester of pregnancy related to pregnancy outcome?

Subsequent to the assessment of associations between uterine artery vascular resistance and pregnancy outcome, research should be directed towards detection of potentially modifying determinants of vascular resistance during pregnancy. Therefore, using data obtained in the same 393 women, two secondary research questions were addressed:

1) Are serum calcium levels and dietary calcium intake associated with blood pressure, pulsatility index and/or pregnancy outcome?

2) Are total and HDL cholesterol related to blood pressure, pulsatility index and/or pregnancy outcome?

Chapter 2 presents a discussion of the literature uteroplacental Doppler studies and a brief overview of the histological changes occurring during pregnancy. In chapter 3 the association of uterine artery PI with pregnancy outcome is reported. Chapter 4 addresses the relation of blood pressure, PI and pregnancy outcome with calcium and cholesterol. In chapter 5 general conclusions of the presented studies are given.

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CHAPTER 2

UTERINE BLOOD FLOW VELOCIMETRY: A LITERATURE REVIEW

2

UTERINE BLOOD FLOW VELOCIMETRY: A LITERATURE REVIEW

The introduction of Doppler ultrasound technology has made it possible to assess the arterial bloodflow of the uteroplacental circulation non-invasively. The application of Doppler ultrasound to measure uteroplacental flow was first reported by Campbell et al[1] and they concluded that the use of Doppler ultrasound might be a new technique to give early warning of an impaired uteroplacental circulation. This report was followed by many others, studying uteroplacental flow velocity waveforms (FVW) in both normal[2-19] and complicated[2,20,24,27-30,37] pregnancies and investigating the predictive capabilities of uteroplacental flow velocity waveforms [20,21,22,25,26,31-36]. Tables 2.1 and 2.2 summarize the main characteristics and results of these studies. The discrepancies between the findings may result from methodological issues. In the following paragraphs the available studies will be discussed with special emphasis on methodological aspects.

2.1 Methodological aspects

Uteroplacental flow velocity waveforms in normal pregnancy as a measure of vascular resistance have been investigated in both cross sectional and longitudinal studies in all three trimesters of pregnancy (table 2.1). During the first trimester of pregnancy both decreasing[6,12,13,16-19] and unaltered[4,14] uteroplacental FVW indices have been shown, whereas virtually all studies report a decline during the second trimester[1-12,15], which last until 20 weeks[7], 24 weeks[5,6,12] or 26 weeks gestation[4]. Several studies, however, report this decline to continue until term[3,8-11].

The relationship between uteroplacental FVW indices and pregnancy complications has been investigated in a number of prospective follow-up studies, which provided inconsistent results (table 2.2). Several authors found uteroplacental FVW indices to be associated with pregnancy-induced hypertension (PIH) with or without proteinuria[1,2] and growth retardation[2,29] whereas others did not[24,37]. To date eleven screening studies for complications of pregnancy have been published[20, 21,22,25,26,31-36] with a large range in predictive values of the various complications (table 2.2).

Uteroplacental FVW measurements

Measurements of uteroplacental FVW's have been performed by either duplex pulsed wave (PW) or continuous wave (CW) devices. PW equipment has been claimed to adequately identify uteroplacental vessels due to exact depth selection, whereas multiple vessels in the path of the CW beam may lead to erroneous measurement[21]. However, in several duplex PW Doppler studies vessel identifica-

tion was based on waveform patterns, which were regarded as typical for the vessel under study[21,22]. Furthermore, colour flow Doppler demonstrates that ultrasonographic images resembling a blood vessel may not always correspond to colour flow encoded shades of red and blue. In any case, comparitative studies have shown consistent results for both CW and PW devices[3,38,39].

Although each blood vessel has a characteristic waveform, the uterine-arcuate-radial-spiral artery circulation is rather complex and gives rise to difficulties with respect to insonation of the uteroplacental arteries. No agreement exists as to which artery or arteries best reflect vascular resistance of the uteroplacental vascular bed as a whole and as to whether vascular impedance is similarly affected in different parts of the vascular tree under pathological conditions. It has been shown that peripheral vascular resistance is lower in the distal arcuate arteries than in the proximal uterine arteries[4,40,41], and vascular resistance in the vessels on the placental side of the uterus tends to be lower than on the opposite side[41-43].

The uteroplacental FVW corresponds with the red blood cell velocities during one cardiac cycle and analysis of this waveform provides an estimate of resistance to blood flow in a vessel. The methods available for analysis of waveforms all depend on a pulsatile blood flow. The S/D ratio[44] and the resistance index (RI)[45] are calculated from peak systolic and end-diastolic shifts, whereas the pulsatility index (PI)[46] involves the measure of the entire waveform. Campbell et al[1] calculated the frequency index profile (FIP) which is based on normalisation of the entire waveform. Although the S/D ratio is the simplest index the values are not normally distributed[47]. Furthermore, additional information in the waveform shape (i.e., a notch) is lost in the S/D ratio and RI.

Regardless of the index used, definitions of abnormal FVW's differ substantially among the various Doppler studies. Several studies used absolute cut-off levels[20,21,22,25,26,28,31,32,36] whereas others used a cut-off >95th centile for gestation[27,30,34,37] or 2 standard deviations or 1.96 standard error of the mean above the mean for a normal population[23,24]. The choice of cut-off level should eventually be determined by the true positive and negative findings and hence the predictive values shown in follow-up studies.

Study population

The selection of subjects is an important methodological issue, differences in which may potentially lead to differences in results. The uteroplacental Doppler studies on the prediction of adverse pregnancy outcome have been performed in various populations including uncomplicated pregnancies[21,26-28,31,33,35], pregnancies at risk of or complicated by hypertensive disorders[1,2,20,22-25,29,30,37], growth retardation[36] or both[1,32]. This has considerable implications on the estimates of prevalence of adverse pregnancy outcome, ranging from 2%[31] to 37%[22] for PIH with or without proteinuria, from 1%[31] to 38%[36] for growth retardation, and from 2%[31] to 31%[25] for combinations of complications. Hence the predictive values, which depend upon prevalence, differ substantially among the different studies. In populations with a high prevalence of PIH the positive predictive value will be higher[22] than that applicable to a normal population[31]. In view of the large differences in prevalence of the various pregnancy complications the predictive values reported in the Doppler studies cannot be directly compared to one another.

Table 2.1 Summary of uteroplacental Doppler studies in normal pregnancy.

first author (ref.)	Design	N	gestational age	Method	Vessel	Site	Index	conclusions
Campbell (1)	CS	30	2nd, 3rd term	PW	AA	L or R	FIP, RI	both ratios constant
Cohen-Overbeek (2)	L	10	14-20 wks	PW	AA	P	FIP	significant decline
Trudinger (3)	L	12	20-40 wks	CW	AA	P	D/S	non-significant increase
Schulman (4)	CS	79	1-40 wks	CW	UV	mean L/R	S/D	constant until 10 wks, significant decline in 2nd term constant after 26 wks
Pearce (5)	L	34	16-40 wks	PW	AA	P and NP	RI	non-significant decline significant decline
Deutinger (6)	CS	71	7-40 wks	PW	UA	mean L/R	PI, S/D	both ratios significant decline until 24 wks
McCowan (7)	L	15	16-40 wks	CW	UA	mean L/R	PI, S/D	significant decline until 20 wks
Al Ghazali (8)	CS	271	16-42 wks	PW	UA	?	D/S	non-significant increase
Mulders (9)	L	41	18-38 wks	CW	UA	mean L/R	PI, S/D	both ratios non-significant decline
Gudmundson (10)	CS	125	20-42 wks	PW	AA	P	PI, S/D	both ratios significant decline
Baumann (11)	L	21	16-delivery	PW	AA	L	RI	significant (?) decline
Rudelstorfer (12)	CS	42	8-40 wks	PW	UA	mean L/R	PI, S/D	significant decline until 3rd term
Stabile (13)	L	30	6-16 wks	PW	UA	L or R	RI	significant (?) decline
Schaaps (14)	CS	98	6-12 wks	PW	UA	mean L/R	RI	constant
Bewley (15)	CS	993	16-24 wks	CW	UA, AA	P and NP	RI	significant decline
Den Ouden (16)	CS	83	8-13 wks	CW	UA	mean L/R	PI, RI	significant decline
Jurkovic (17)	CS	45	4-18 wks	colour	UA	mean L/R	PI, RI	significant decline
Jaffe (18)	CS	50	5-9 wks	colour	Tb	mean L/R	RI, S/D	significant (?) decline
Arduini (19)	CS	282	7-16 wks	colour	UA, AA	mean L/R	PI, S/D	significant decline

CS=ross sectional, L=longitudinal, PW=pulsed wave, CW=continuous wave, AA=arcuate artery, UA=uterine artery, Tb=throphoblast, L=Left, R=right, P=placental, NP=non-placental, FIP=frequency index profile, RI=resistance index, D/S=diastolic/systolic ratio, S/D=systolic/diastolic ratio, PI=pulsatility index

Tabel 2.2 Summary of uteroplacental Doppler studies in complicated pregnancies and predictive abilities of uteroplacental Doppler studies.

first author (ref.)	N	Population	Gestational age	method	vessel	site	index	abn. FVW	End points	SE (%)	SP (%)	PPV (%)	NPV (%)	PVL (%)
Campbell (1)	31	PIH +/- proteinuria and/or IUGR	± 35 wks	PW	AA	P	FIP	2 point outside FIP	PIH + proteinuria 1-min Apgar Mean BW (ratio) Cesarean section					
Cohen-Overbeek (2)	53	PIH +/- proteinuria	34 wks	PW	AA	P	FIP	2 points outside FIP	1-min Apgar Mean BW (ratio) SGA Cesarean section					
Fleischer (20)	71	hypertensive disorders	3rd term	CW	UA	mean L/R	S/D	≥2.6 ≥2.6 + notch	Delivery <37 weeks + BW <2500 gm	81 87	90 95	86 93	86 91	44
Campbell (21)	126	uncomplicated	16-18 wks	PW	AA	P	RI	>0.58	PIH + SGA + FD	68	69	42	87	25
Arduini (22)	60	risk of PIH	18-20 wks	PW	AA	L + R	RI	≥0.57	PIH PIH + SGA	64 63	84 71	70 25	80 90	37 13
McCowan (22)	12	uncomplicated PIH +/- proteinuria	day of delivery	CW	UA	P + NP	PI S/D	mean+1.96SEM mean+1.96SEM	SGA					
Gudmundson (24)	58	PIH +/- proteinuria	within 19 days of delivery	CW	AA	P	PI	mean + 2SD	SGA FD					
Mulders (25)	145	uncomplicated suspected IUGR	within 45 days of delivery	CW	UA	mean L/R	PI	<32 wks: ≥1.02 ≥32 wks: ≥0.92	SGA and/or FD	49 30	83 90	53 58	80 26	28 31
Steel (26)	200	uncomplicated	18-20 wks 24 wks	AA	L + R	CW	RI	≥0.58 on one side	PIH PIH + SGA PIH PIH + SGA	41 45 29 33	64 65 92 91	9 18 25 40	66 87 93 89	9 15 9 15
Hanretty (27)	291	uncomplicated	26-30 wks	CW	UPA	L or R	S/D	>95th centile						
Schulman (28)	255	uncomplicated	20-26 wks	CW	UPA	mean L/R	S/D	>2.7	SGA PIH +/- proteinuria					
Janbu (29)	97	uncomplicated PIH +/- proteinuria	within 20 days of delivery	PW	AA	L + R	S/D		SGA					

contined....

tabel 2.2 (cont'd)

first author (ref.)	N	Population	Gestational age	method	vessel	site	index	abn. FVW	End points	SE (%)	SP (%)	PPV (%)	NPV (%)	PVL (%)
Trudinger (30)	37	PIH +/- proteinuria	mean 1.6 days before delivery	CW	UPA	P	S/D	>95th centile	SGA Perinatal death NICU admission					
Steel (31)	1014	uncomplicated	24 wks	CW	AA	L + R	RI	≥0.58 on one side	PIH	39	91	25	95	8
									PIH + proteinuria	63	89	10	99	2
									PIH + IUGR	100	90	13	100	2
									SGA	33	91	27	93	1
									preterm delivery	75	90	15	99	3
Jacobson (32)	93	risk of PIH and IUGR	24 wks	PW	UA	L + R	RI	≥0.58 on one side	PIH	44	33	63	73	29
									PIH + prot.	67	17	63	95	10
									SGA	71	33	68	91	1
Bewley (33)	977	uncomplicated	16-24 wks	CW	UA/AA	P + NP	AVRI	>95th centile	PIH	10	95	10	95	5
									PIH + proteinuria	24	95	20	96	5
									SGA	15	96	35	88	13
Newnham (34)	535	medium risk	18 wks	CW	UA	P	S/D	>95th centile	FH	10	95	19	90	10
									SGA	6	95	13	90	10
									FH + SGA	13	95	4	99	2
			24 wks						FH	24	94	30	92	10
									SGA	6	92	5	93	7
									FH + SGA	-	92	-	99	1
			28 wks						FH	2	95	5	90	9
									SGA	7	96	15	12	10
									FH + SGA	13	96	5	98	2
			24 wks						FH	3	95	5	91	9
									SGA	7	96	16	92	8
									FH + SGA	14	96	5	99	2
Harrington (35)	2437	uncomplicated	20 wks	CW	UA	L + R	RI	>95th centile	PIH + proteinuria	76	86	13	99	2
			24 wks						PIH + proteinuria	76	96	35	99	2
			26 wks						PIH + proteinuria	74	97	44	99	2

contined....

first author (ref.)	N	Population	Gestational age	method	vessel	site	index	abn. FVW	End points	SE (%)	SP (%)	PPV (%)	NPV (%)	PVL (%)
Kay (36)	48	suspected IUGR	26-41 wks	PW	AA	L + R	S/D	≥2.0 both arteries	SGA	17	93	60	65	38
Kofinas (37)	123	Hypertensive disorder	within 10 days of delivery	CW	UA	mean L/R	S/D RI	95th centile	SGA, 5 min Apgar Preterm delivery Cesarean delivery perinatal death NICU admission					

PIH=pregnancy-induced hypertension, IUGR=intrauterine growth retardation, PW=pulsed wave, CW=continuous wave, AA=arcuate artery, UA=uterine artery, UPA=uteroplacental artery, L=Left, R=right, P=placental, NP=non-placental, FIP=frequency index profile, RI=resistance index, D/S=diastolic/systolic ratio, S/D=systolic/diastolic ratio, PI=pulsatility index, AVRI=averaged resistance index, SGA-small-for-gestational age, NICU=neonatal intensive care unit, SE=sensitivity, SP=specificity, PPV=positive predictive value, NPV=negative predictive value, PVL=prevalence.

2.2 Physiological mechanisms

In order to accommodate the large increase in uteroplacental blood flow during pregnancy the spiral arteries of the non-pregnant uterus are converted into uteroplacental arteries as a result of trophoblast invasion[48]. The vascular adaptations of the spiral arteries occur in two separate waves[49] of which the first takes place during the first 10 weeks of gestation. In this first wave the decidual spiral arteries are converted, whereas during the second wave, which starts at 14-16 weeks gestation and lasts for 4-6 weeks, the intramyometrial parts of the spiral arteries are invaded by trophoblast. Due to progressive distension of the arteries uteroplacental vascular resistance is reduced and, in addition, the arteries have become insensitive to circulating pressor agents.

In pregnancies complicated by preeclampsia the second wave of trophoblast invasion fails to develop properly [49], the arteries remain responsive to vasomotor influences and acute atherosis may develop[50] leading to decreased perfusion of the intervillous space. Similar observations are made in approximately half of the pregnancies complicated by intrauterine growth retardation[51,52].

Several studies report declining indices from the uteroplacental arteries until about 26 weeks gestation[4,5,6,7] to remain virtually unchanged thereafter. It was suggested that the uteroplacental waveforms reflect the changes in vascular resistance as a result of trophoblast invasion, which is confirmed by the association between Doppler findings of increased uteroplacental vascular resistance and histomorphological findings[53,54]. However, biochemical factors also exert their effect on uteroplacental circulation and vascular resistance. Disturbance of the physiological balance between vasodilatator substances, such as prostacyclin, and vasoconstrictor substances (i.e., tromboxane A_2 and angiotensine II) may lead to increased vascular resistance of the uteroplacental bed. However, the relative importance of both morphological and biochemical mechanisms and their functional interrelationship is largely unknown.

2.3 Conclusions

Results of uteroplacental Doppler studies in both normal and complicated pregnancies may be influenced by a number of methodological characteristics. These include insonation sites along the uteroplacental vascular tree, choice of indices to describe flow velocity waveforms and cut-off points for abnormal waveforms. However, despite differences in study design a major drop in vascular resistance generally seems to occur during the first half of normal pregnancy until approximately 26 weeks. Thereafter, only slight decreases, if any, are observed until term.

In the interpretation of the results of follow-up studies a major problem resides in the selection of study populations. Subjects at risk of hypertensive disorders or fetal growth retardation display a higher prevalence of adverse pregnancy outcome compared to subjects with normal uncomplicated pregnancies. This will lead to large differences in predictive values and as a result comparison of data from different studies is questionable.

Trophoblast invasion of the spiral arteries as well as failure of this process have been suggested to correlate with the shape of the uteroplacental flow velocity waveform. However, it seems likely that both biochemical and morphological

mechanisms are involved in the regulation of the uteroplacental circulation. The uteroplacental waveform is determined by a heterogeneous set of factors.

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CHAPTER 3

DOPPLER ULTRASOUND AND ADVERSE PREGNANCY OUTCOME

3.1

THE PREDICTIVE VALUE OF UTERINE ARTERY FLOW VELOCITY WAVEFORMS IN SPONTANEOUS ABORTION IN OLDER WOMEN

Abstract

Women of advanced maternal age are considered to be at a substantially increased risk of spontaneous abortion. The increased spontaneous abortion rate in older women might be associated with a defective vascular response to placentation, which might lead to fetal ischaemia and fetal dead. Doppler velocimetry of the uterine artery allows non-invasive assessment of uteroplacental hemodynamics.

The association of uterine artery Doppler flow velocity waveforms with the risk of spontaneous abortion was examined in 393 women aged 35 years and older in the first trimester of pregnancy. Twenty women aborted spontaneously; 10 pregnancies were terminated because of chromosomal anomalies. Maternal age and gestational age at intake were significantly associated with the spontaneous abortion rate ($p=0.01$ and $p=0.001$, respectively). Uterine artery PI values declined significantly during the first trimester ($p=0.001$). However, no association was found between uterine artery PI values and spontaneous abortion rate. PI, maternal age and gestational age at intake were not essentially different between women who aborted spontaneously before or after chorionic villus sampling. No association was found between PI, maternal age, and gestational age at intake and genetic abortion rate.

These findings indicate that uterine artery blood flow velocity waveforms as expressed by the pulsatility index, bear no relationship with spontaneous abortion.

Introduction

The reported spontaneous abortion rate among clinically recognised pregnancies is between 12% and 15%[1]. However, when fetal viability is taken into account the incidence is as low as 2.0% - 3.3%[2]. Moreover an inverse relation exists between gestational age at which fetal viability is established and spontaneous abortion[3]. The risk of both chromosomally normal and abnormal abortions increases substantially with advancing maternal age[4]. A steady rise in early fetal loss in previously viable pregnancies has been demonstrated, from 1.9% at age 35-36 years to 10.9% at 40 years and older[3]. The exact mechanism behind chromosomally normal abortions in both younger and older women remains unexplained, but a defective maternal vascular response to placentation leading to fetal ischaemia and possibly death has been suggested[5].

Normal fetal growth and development depend on adequate perfusion of the uterus. Doppler velocimetry has become widely accepted as a non-invasive

technique to assess fetal and uteroplacental hemodynamics[6].

This study was undertaken to examine whether measurement of blood flow in the uterine artery by means of Doppler velocimetry can adequately predict spontaneous abortion in older women.

Subjects and methods

Between january 1989 and may 1991, 393 women were recruited from the Prenatal Diagnosis Unit at the time of the intake visit for a late first trimester chorionic villus sampling. Inclusion criteria were maternal age 36 years or older at 20 weeks of gestation, a viable singleton pregnancy and gestational age not beyond 11 weeks. Women who suffered from diabetes, hypertension or other cardiovascular disease were excluded.

Each subject received a written outline of the study and verbal consent was obtained in each instance. General characteristics and data on known potential risk factors were documented (table 3.1).

Uteroplacental waveforms were obtained using 4 MHz continuous-wave doppler ultrasound equipment ('Doptek'). Doppler waveform recordings were performed with women in the semirecumbent position. The Doppler probe was oriented along the transverse axis of the uterus, slightly medially from the external iliac artery with the Doppler beam aimed at the lateral wall of the uterus. The flow velcity wavefroms were thus obtained from the mainstem uterine arteries from both sides of the uterus. On either side the mean pulsatility index[8] (PI) was calculated from three consecutive wavefroms. The PI used for analyses always presented the average of the left and right uterine arteries, which, when taken together, are believed to adequately represent total uterine perfusion.

The primary outcome in this study was spontaneous abortion. Continuing pregnancies were followed up until 26 weeks of gestation.

Statistical analysis consisted of univariate analysis using the chi-square test and unpaired t-tests for comparing the general characteristics (table 3.1) from women who spontaneously aborted with data from the controls. Multiple linear and logistic regression was used to investigate the association between spontaneous abortion and the Doppler data adjusting for potentially confounding variables (table 3.1).

Results

Twenty pregnancies in which fetal viability was previously confirmed by ultrasound, resulted in spontaneous abortion before 20 weeks of gestation (mean gestational age=12.5 wks, sd=3.7). Ten pregnancies were terminated because of chromosomal abnormalities (mean gestational age=14 wks, sd=0.3).

A significantly inverse association was observed between spontaneous abortion and gestational age at intake (odds ratio=0.4, se=0.2, p=0.0001). The spontaneous abortion rate increased significantly with advancing maternal age (odds ratio=1.4, se=0.1, p=0.01, adjusted for gestational age at intake). The percentage of women which aborted spontaneously rose from 1.6% at age 35-36 years (n=2), to 5.2% at age 37-39 years (n=11) and 12.3% in the group aged 40 years and older (n=7).

Table 3.1 Baseline data on maternal characteristics at entry into the study*

Characteristic	Study population	Spontaneous abortion	Genetic abortion
No. of subjects	393	20	10
Age (yrs)			
mean ± sd	37.6 ± 1.8	38.8 ± 2.0✡	37.2 ± 1.5
range	35 - 44	36 - 43	36 - 43
Parity			
mean ± sd	1.3 ± 1.0	1.6 ± 1.0	1.4 ± 1.0
range	0 - 7	0 - 3	0 - 3
Gestational age (wks) at enrolment			
mean ± sd	9.5 ± 1.1	8.5 ± 1.2✡✡	9.6 ± 0.8
range	6.0 - 11.0	6.0 - 10.5	8.5 - 10.5
Previous spontaneous abortions			
1-2	117 (30)	7	4
≥3	9 (2)	1	1
Previous induced abortions	29 (7)	2	-

* Data shown are numbers of women, percentages are shown in parentheses
✡ $p<0.05$ and ✡✡ $p<0.005$ for the comparison with continuing pregnancies

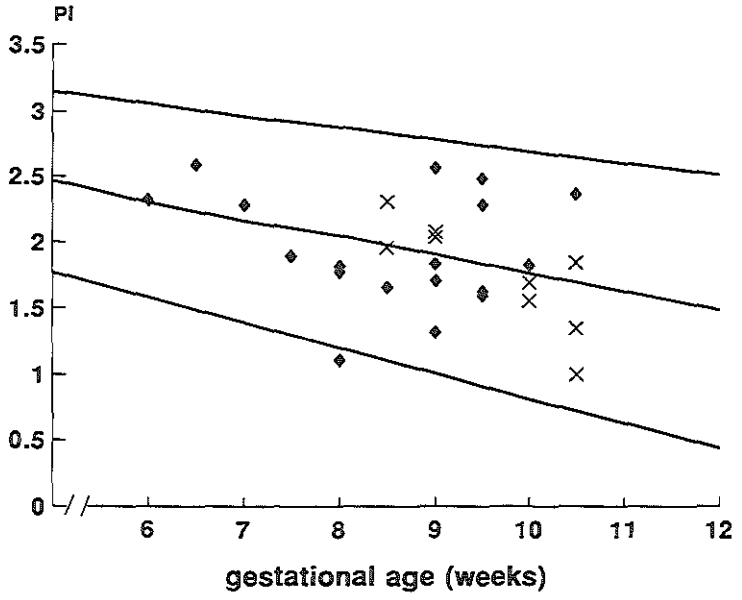

Figure 3.1 Regression line and 95% percent confidence intervals of the association between PI and gestational age. The index values from spontaneous aborted cases are symbolized by ♦, genetic aborted cases are represented by the symbol x.

Technically acceptable uterine artery flow velocity waveform recordings from both uterine arteries were obtained in 91% of the total study population. Gestational age at enrolment in women with unsuccessful uterine artery waveform recordings was significantly lower than in women with successful recordings (8.9 wks vs 9.4 wks, p=0.002). In two cases in which waveform recordings failed, spontaneous abortion occurred.

A significant decline in PI was observed with advancing gestational age (coefficient of linear regression=-0.1, se=0.02, p=0.0001; figure 3.1). PI values from the 18 spontaneously aborted cases were all situated within the normal range and no significant association between PI and spontaneous abortion was observed (p=0.65).

In 14 women spontaneous abortion occurred before chorionic villus sampling. No differences in PI, maternal age and gestational age at intake were observed between these women and those who aborted after chorionic villus sampling.

In the ten pregnancies that were terminated because of chromosomal abnormalities no association was found between these genetic abortions and maternal age, and gestational age at intake. The same applied to the PI (figure 3.1).

Discussion

The results of our study indicate that no relationship exists between first trimester uterine artery flow velocity waveform measurements and spontaneous abortion.

Our study only included women of advanced maternal age who are at a substantial risk of chromosomally determined fetal loss. We do not believe this has introduced bias, since the maternal age dependent rise in fetal loss results from both an increase in uterine dysfunction, particularly after the age of 36 years[5], and a diminished oocyte quality[9]. The data from the ten genetic abortions seems to support this since no relation existed between these terminated pregnancies and maternal age. However, this finding has to be interpreted with care as the number of genetic abortions was rather small.

The present study confirms a decline in spontaneous abortion rate with advancing gestational age. Women entering the study at weeks 6 and 7 have a high initial abortion risk during week 9 and 10. The exclusion of all non-viable pregnancies might explain the decreasing abortion rate in women enroled into the study at 9 weeks and later.

The significant decline in PI observed during the first trimester is believed to reflect a decrease in vascular resistance due to trophoblast induced alterations of the spiral arteries between the sixth and tenth week of gestation[10]. In women with spontaneous abortion not attributable to a chromosomal anomaly, absent trophoblastic invasion in the spiral arteries has been detected with a subsequently abnormal uteroplacental arterial response to placentation[5,11]. However, in our study population, the PI in women who aborted did not differ from the controls. Because several fetal losses in our study undoubtedly occurred as a result of chromosome abnormality we may not have had sufficient precision to detect PI differences. Nevertheless, PI values in women who aborted show no tendency to deviate from the normal pattern observed in normal pregnancies suggesting that the age-related increase in spontaneous abortion is attributable to oocyte quality rather than uterine dysfunction.

It can, however, also be postulated that although PI values parallel the haemo-

dynamic changes occurring in early pregnancy, maternal uterine haemodynamic changes as assessed by means of Doppler velocimetry can not be attributed solely to histological phenomena in the placental bed[5].

In the light of these results, the physiological factors that underlay the flow velocity waveform warrant further investigation.

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3.2

EARLY UTERINE ARTERY DOPPLER VELOCIMETRY AND THE OUTCOME OF PREGNANCY IN WOMEN AGED 35 YEARS AND OLDER

Abstract

In this prospective study of 352 women of 35 years and older the association between first and second trimester uterine artery Doppler flow velocity waveforms, expressed as pulsatility index, and pregnancy complications was examined.

Pulsatility index (PI) values at 12-13 weeks of gestation were significantly associated with development of hypertensive disorders, SGA and gestational diabetes, with gestational age adjusted risks exceeding 4, 2 and 8 for women with PI values in the highest quartile (>1.67) of the PI distribution when compared with the lowest quartile of the PI distribution (<1.24). At 23-27 weeks gestation uterine artery PI values were found to be associated with preterm delivery with a gestational age adjusted risk of 10.6 for women with PI values in the highest quartile of PI (>1.24) when compared with PI values in the lowest quartile of the PI distribution (<1.09). No associations existed between uterine artery PI, antepartum haemorrhage and caesarean section rate.

The risk estimates for any of the outcome parameters were not affected by maternal age.

The results of this study indicate that an increased risk of hypertensive disorders, SGA and gestational diabetes is associated with Doppler velocimetry detectable changes in the uterine artery as early as the first trimester of pregnancy, whereas similar changes in the late second trimester of pregnancy are related to an increased risk of preterm delivery.

Introduction

Advanced maternal age is known to contribute to an increased incidence of pregnancy complications[1,2], possibly related to a decreased uterine function[3,4], which might result in impaired uterine perfusion. The uteroplacental circulation is influenced by morphological[5] and biochemical[6] factors, but the relative importance of these factors in adaptive changes occurring during pregnancy is still largely unknown. Disturbances in the physiological balance between fore-mentioned mechanisms affecting uteroplacental circulation may result in increased uteroplacental vascular resistance with subsequent decreased placental and fetal blood flow.

In normal pregnancy a fall in uteroplacental vascular resistance has been demonstrated from early pregnancy until approximately 24 weeks' gestation[7] most likely as a result of trophoblast invasion into the spiral arteries[8]. Pregnancies in which this invasion fails may be complicated by adverse perinatal outcome[9,10].

In this study the association between Doppler uterine artery waveforms recorded in first and second trimester pregnancies of women of advanced maternal age and pregnancy complications is investigated with special emphasis on hypertensive disorders of pregnancy, the small-for-gestational-age infant, preterm delivery and gestational diabetes.

Subjects and Methods

Between June 1989 and June 1991 a prospective Doppler study of the uteroplacental circulation was conducted in women of 35 years and older. Pregnant women referred to the prenatal diagnosis unit of the academic hospital Rotterdam Dijkzigt for chromosome analysis by late first trimester transabdominal chorionic villus sampling (CVS) were invited to participate. The study was approved by the local Ethics Review Board and informed consent was obtained from each participant.

Criteria for enrolment included: diastolic blood pressure at recruitment below 85 mmHg, no history of hypertension, cardiovascular disease or diabetes, a viable singleton pregnancy not beyond 11 weeks, and maternal age 36 years or older at 20 weeks of gestation.

All women were seen on three separate occasions: i) at recruitment for CVS between 7 and 11 weeks of gestation (measurement 1); ii) at CVS at 12-13 weeks of gestation (measurement 2) and (iii) between 23 and 27 weeks of gestation (measurement 3).

At the initial visit general characteristics and data on known risk factors were collected by means of an interview (table 3.2.1), and fetal viability and gestational age were confirmed by ultrasound. Uterine artery Doppler flow velocity waveforms and maternal blood pressure were recorded at each visit with the woman in the semi-recumbent position. Umbilical artery flow velocity waveforms were only obtained at 23-27 weeks. Flow velocity waveforms from the uterine and umbilical artery were recorded by means of a 4 MHz continuous wave Doppler ultrasound system (Doptek, Chichester ,UK). The method of waveform recordings and analysis has been presented previously[11]. The average of the left and right uterine arteries is represented by the mean pulsatility index (PI)[12]. Blood pressure was recorded on the right arm with a random zero sphygmomanometer; the values reported here represent the mean of two readings taken at a one minute interval. All measurements were performed by one investigator (HvdE).

A total of 393 women were enroled into the study. Thirty pregnancies (7.6%) resulted in spontaneous abortion (n=20) or were terminated because of chromosomal abnormalities (n=10). A total of 11 women (3%) was lost to follow-up, leaving 352 women for further analysis. The baseline characteristics of the 11 women were similar to the rest of the cohort. One woman was hospitalized during the third visit and missing data from this subject were included in the analysis as missing values. One intrauterine death occurred at 26 weeks of gestation. Furthermore one maternal death resulting from amniotic embolism at 41 weeks of gestation was reported.

Follow-up was assessed by means of a questionnaire sent to referring obstetricians and midwives. Hypertensive disorders considered are i) pregnancy-induced hypertension (PIH), defined as diastolic BP ≥90 mmHg or an increase of diastolic BP of 20 mmHg or more on two separate occasions after 16 weeks

gestation; ii) preeclampsia defined as PIH with proteinuria >500 mg/24h or urine semi-quantitative dipstick testing of ++ or more; iii) eclampsia. The rates of preterm (<37 completed weeks of gestation), and small-for-gestational age (SGA, birth weight <10th centile for gestational age and sex[13]) infants were computed for live births only. The rates of gestational diabetes, antepartum haemorrhage (bleeding from the genital tract after 28 weeks of gestation), and delivery by cesarean section were also assessed.

The risk for each of the outcome categories assessed was estimated for every increase in PI by 0.1. In addition, PI values were analyzed after dividing the sample into four groups according to quartiles for distribution of PI values with the lowest quartile of PI serving as a reference group. Analysis was performed by multiple linear and logistic regression analysis; the odds ratios derived from logistic regression analysis were used as an approximation of relative risk. Results are presented with 95% confidence intervals and two-sided p-values when appropriate.

In the analyses performed numbers do not all add up to the total number of women available due to slight differences in the number of missing values for the various parameters.

Table 3.2.1 Baseline characteristics of 352 women of 35 years and older.

Risk Factor	Study population N = 352
Maternal age (yrs)	
mean ± sd	37.5 ± 1.8
range	35 - 44
Body mass index (Kg/m^2)	
mean ± sd	23 ± 4
range	17 - 39
Cigarette smoking (%) (smoked during index pregnancy vs did not smoke)	24
Alcohol use (%) (alcohol use during index pregnancy vs no alcohol use)	31
Nulliparity (%)	27
Previous spontaneous abortion (%)	31
Previous induced abortion (%)	7
Previous intrauterine death (%)	3
History of PIH with or without proteinuria (%)	16
History of gestational diabetes (%)	4

Values are means ± sd or percentages.

Results

The success rate in obtaining technically acceptable uterine artery velocity waveforms was 91%, 97% and 100% at measurement points 1, 2 and 3, and 100% in the umbilical artery at measurement 3. Mean gestational age at the three measurements was 9.2 (sd 0.9), 12.5 (sd 0.5) and 24.6 (sd 0.8) weeks, respectively. Gestational age was controlled for in all further data analyses. Mean maternal age was 37.5 ± 1.8 years.

A significant increase in uterine artery PI with advancing maternal age was observed at 23-27 weeks gestation (coefficient of linear regression 0.8, 95% confidence interval (CI)=0.1-1.7). Pregnancy outcome was not associated with maternal age.

Hypertensive disorders (n=37)

At measurements 1 and 2 the risk of subsequent development of hypertension was positively associated with diastolic blood pressure (relative risk (RR)=1.1 per mmHg, 95% CI=1.1-1.2); no association was found with maternal age.

Also at measurement 2 the risk of hypertension was positively associated with PI (RR=1.2 per 0.1 PI, 95% CI=1.1-1.4, after adjustment for diastolic blood pressure and gestational age) and the risk of hypertension increased significantly with increasing quartiles of PI (table 3.2.2). A relative risk of 4.2 (95% CI=1.3-12.1) was observed for women with PI values in the highest quartile (i.e. PI above 1.67) as compared with women with PI values in the lowest quartile (i.e. PI below 1.24).

In six women hypertension had already been diagnosed at the time of the third measurement and these women presented with higher uterine artery PI values compared with non-hypertensive women (1.54 vs 1.11, p=0.04 after adjustment for gestational age).

Hypertension was classified according to severity and presence of proteinuria (e.g preeclampsia). Pregnancy-induced hypertension (PIH) was diagnosed in 25 women, preeclampsia in 11 women and eclampsia in one woman. PI values from women developing preeclampsia were not essentially different from any PI values from women developing PIH. At measurement 1 a non-significant trend was visible and at measurement 3 no significant association was observed between PI values and the development of preeclampsia. In the only woman who developed eclampsia a PI value of 1.53 was recorded at measurement 3.

SGA infants (n=36)

The risk of delivering a SGA infant increased significantly with increasing uterine artery PI values (RR=1.1 per 0.1 PI, 95% CI=1.0-1.2) at measurement 2. In 6 cases this was preceded by the development of PIH, however, risk estimates were similar for women with a combination of SGA and PIH and SGA alone. The risk of delivering a SGA infant was 2.4 (95% CI= 0.8-6.6) in women with uterine artery PI values in the highest quartile of PI (>1.67) when compared with uterine artery PI values in the lowest quartile of PI (<1.24) (table 3.2.2)

Preterm delivery (n=19)

A significant association was observed between risk of preterm delivery and the rise in uterine artery PI (RR=1.4 per 0.1 PI, 95% CI=1.2-1.7) at measurement 3. The risk of preterm delivery increased significantly across the subsequent quartiles of uterine artery PI at this stage of pregnancy (test for trend p=0.007; table 3.2.2). In women with uterine artery PI values in the highest quartile of PI (>1.24) the risk of preterm delivery was ten fold higher than in women with uterine artery PI values in the lowest quartile of PI (<0.96). In three women preterm delivery was

Table 3.2.2 Relative risks of hypertensive disorders, preterm delivery, SGA infants, and gestational diabetes by quartiles of PI for each measurement.

	PI*	Total N	Hypertensive disorders		SGA Infants		Prematurity		Diabetes	
			N	RR (95% CI)**	N	RR (95% CI)**	N	RR (95% CI)**	N	RR (95% CI)**
	0.80-1.52	80	7	1.0†	10	1.0†	3	1.0†	4	1.0†
	1.53-1.78	79	8	1.2 (0.4-3.5)	5	0.5 (0.2-1.5)	5	1.7 (0.4-7.5)	3	0.8 (0.2-3.5)
7-11 weeks	1.79-2.07	81	9	1.6 (0.5-4.2)	4	0.4 (0.1-1.2)	4	1.4 (0.3-6.2)	5	1.3 (0.3-4.8)
of gestation	2.08-4.25	80	9	1.6 (0.5-4.2	13	1.4 (0.6-3.3)	4	1.4 (0.3-6.2)	5	1.3 (0.3-4.9)
	0.67-1.24	86	6	1.0†	6	1.0†	2	1.0†	1	1.0†
12-13 weeks	1.25-1.41	84	7	1.0 (0.3-3.3)	8	1.4 (0.3-5.9)	7	3.8 (0.8-18.9)	5	5.3 (0.5-52.8)
of gestation	1.42-1.66	85	8	1.4 (0.4-4.4)	5	0.8 (0.2-2.8)	4	2.1 (0.4-11.7)	1	1.0 (0.1-16.4)
	1.67-2.67	86	14	4.2 (1.3-12.1)✡	13	2.4 (0.8-6.6)	6	3.1 (0.6-16.1)	8	8.7 (1.1-71.3)✡
	0.55-0.96	87	10	1.0†	9	1.0†	1	1.0†	4	1.0†
23-27 weeks	0.97-1.09	87	9	1.1 (0.4-3.0)	8	0.9 (0.3-2.4)	3	3.6 (0.4-35.9)	7	1.8 (0.6-6.4)
of gestation	1.10-1.23	89	6	0.6 (0.6-1.8)	5	0.5 (0.2-1.6)	6	6.6 (0.8-56.9)	5	1.2 (0.3-4.8)
	1.24-1.98	88	6	0.7 (0.2-2.1)	13	1.5 (0.8-3.0)	9	10.6 (1.3-87.2)✡	1	0.2 (0.1-2.2)

* In quartiles, specific for the PI distribution at each of the three measurements. Missing values: 32 (measurement 1), 11 (measurement 2), and 1 (measurement 3). ** Estimated as odds ratios, adjusted for gestational age. † Reference group. ✡ test for trend across the quartiles P<0.05. SGA=small-for-gestational age, RR=relative risk, CI-confidence interval.

associated with PIH. The risk estimates did not alter when PIH was controlled for in the analysis. No association was observed between uterine artery PI and gestational age at the time of delivery (range 33-43 weeks).

Gestational diabetes (n=17)

A significantly elevated risk of developing gestational diabetes with increasing uterine artery PI values (RR=1.2 per 0.1 PI, 95% CI=1.0-1.2) was observed at measurement 2. The risk exceeded 8 for women with uterine artery PI values in the upper quartile of uterine artery PI (RR=8.7, 95% CI=1.1-71.3; table 3.2.2). Gestational diabetes was present in nine women at measurement 3. Uterine artery PI was not significantly different from that in non-diabetic women (1.06 vs 1.11, p=0.5).

No associations existed between antepartum haemorrhage (n=7) and uterine artery PI values. The same holds true for the caesarean section rate (n=40).

Umbilical artery PI showed a positive association with preterm delivery (RR1.4 per 0.1 PI, 95% CI=1.0-1.7, adjusted for gestational age and diastolic blood pressure). A positive association was also observed for the cesarean section rate (RR=1.2 per 0.1 PI, 95% CI=1.1-1.5). However, after adjustment for preterm delivery this association was no longer significant.

Discussion

In this prospective study among women aged 35 years and older the development of hypertensive disorders, SGA and gestational diabetes were significantly associated with increasing uterine artery PI at 12-13 weeks of gestation, whereas the occurrence of preterm delivery was associated with uterine artery PI at 23-27 weeks of gestation.

Before these findings can be accepted some issues need to be addressed. The early referral of women for chromosome analysis because of advanced maternal age allowed us to prospectively study uterine artery flow velocity waveforms in healthy women from the first trimester onwards. A maternal age dependent rise in PI at 23-27 weeks gestation was observed. However, maternal age did not explain the increased risks of hypertensive disorders, SGA, gestational diabetes and preterm delivery associated with raised PI levels. The absence of women younger than 35 may have caused underestimations of the modifying effect of age. However, the possible effect of maternal age on the uteroplacental circulation is believed to occur mainly after the age of 35, with a progressive impact on uterine perfusion after this age[3,4].

Several studies have examined the association between flow velocity waveforms and adverse pregnancy outcome[14,15,16,17]. However, due to a number of methodological differences such as choice of study population, insonation sites, definition of abnormal waveforms and adverse pregnancy outcome no unifying conclusions can be drawn. To date only one other study examined uteroplacental arteries in association with hypertensive disorders and SGA as early as the first trimester[18], and the results of this study are in contrast with ours. This may be due to the fact that in the previous report normal waveform indices were only defined after waveform collection[18].

Pregnancies complicated by hypertensive disorders and/or SGA are associated with a defective or absent first[19] and second wave[20] of trophoblastic invasion. The first wave of trophoblast invasion during the first trimester of pregnancy coincides

with a decrease in uteroplacental vascular resistance at the beginning of the second trimester[21]. Additionally, it was recently reported that in women with hypertension abnormal histomorphological findings correlate strongly with Doppler parameters[8]. The high uterine artery PI values we observed at 12-13 weeks of gestation in women who developed a hypertensive disorder of pregnancy or delivered a SGA infant suggests a defective first wave of vascular response of the uteroplacental arteries. The concept of inadequate trophoblast invasion seems to be supported by the high PI values observed in women who already developed hypertension at 23-27 weeks of gestation. However, the finding of normal flow velocity waveforms at 23-27 weeks of gestation in women who developed a hypertensive disorder or SGA at a later stage of pregnancy might indicate that a limited or absent first wave of trophoblast invasion is not necessarily associated with reduced uterine perfusion in later gestation[19]. Local regulation of uterine blood flow by mutually interrelated local generation of vasoactive agents[22] may attenuate the vasoconstrictor effects and overcome the restricted blood supply to some degree.

Evidence of other mechanisms involved is provided by our observation that women with a high uterine artery PI values at 23-27 weeks of gestation were 10 times more likely to deliver preterm than those with a low uterine artery PI. Steel et al[14] reported a similar observation. In addition, increased umbilical PI values were observed. High PI values may be associated with low uteroplacental blood flow, which is suggested to be a major cause of preterm delivery[23]. Placenta-derived vasoactive substances such as prostacyclin, which is produced in the placenta and myometrium[24] exert an vasodilator effect on vascular smooth muscle with suppression of circulating pressor agents (e.g. angiotensine II and tromboxane A_2) leading to low uteroplacental vascular resistance to flow. Disturbances in the physiological balance between prostacyclin and vasoconstrictor substances may result in a pathologically increased uteroplacental vascular resistance.

Women with high uterine artery PI values at 12-13 weeks gestation are at an increased risk of developing gestational diabetes. Although placental blood flow has been found to be decreased in diabetic pregnancies, no data on uterine Doppler velocimetry in pregnancies complicated by gestational diabetes is currently available. Through which mechanism metabolic factors associated with gestational diabetes might exert an effect on uterine vasculature in early pregnancy is at present unknown. Although, our observations were unexpected, we feel further research is needed to establish the importance of early uterine artery PI in relation to gestational diabetes.

In conclusion, the present study provides evidence that Doppler detectable changes in the uterine artery are associated with subsequent development of pregnancy complications and adverse pregnancy outcome as early as 12-13 weeks of gestation. However, the discrepancy in predictive capacity between the different PI measurements in time and the failure to distinguish for instance between PIH and more severe cases of hypertensive disease during pregnancy appear to reflect a heterogeneity of factors that may influence maternal blood supply and vascular resistance. This should be taken into account when uterine artery velocimetry is used to identify women at risk of adverse pregnancy outcome.

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CHAPTER 4

CALCIUM AND SERUM LIPIDS IN PREGNANCY

4.1

CALCIUM METABOLISM, CALCIUM SUPPLEMENTATION AND HYPERTENSIVE DISORDERS OF PREGNANCY

Introduction

Despite advances in perinatal care, hypertensive disorders of pregnancy remain a major cause of maternal and fetal morbidity, occurring in approximately 5-10% of all pregnancies[1]. Although the cause and pathogenesis are poorly understood a number of primary abnormalities have been postulated, including alterations in systemic hemodynamics, cellular cation metabolism, endocrine function and uteroplacental blood flow[1,2,3,4].

Whereas normal pregnancy is characterised by an increased vascular volume[3] and decreased sensitivity to circulating pressor agents[5], it has been shown that in pregnancies associated with hypertension these changes are absent[3] resulting in enhanced vascular reactivity and increased peripheral vascular resistance.

Vascular smooth muscle tone and hence peripheral vascular resistance, is largely determined by the level of free intracellular calcium[6]. Apart from a direct role in the smooth muscle cell excitation contraction coupling, intracellular free calcium is an important second messenger which controls a number of vasodilating and vasoconstricting processes involving i.e. angiotensine II, prostacyclin and cyclic AMP[5,7]. Although a link between changes in serum calcium concentrations and the development of increased vascular muscle tone is still largely undefined, the potential involvement of intracellular calcium dependent mechanisms in the vascular abnormalities present in hypertensive disorders of pregnancy led to the hypothesis that these complications might be associated with alterations in extracellular calcium metabolism as well. A number of reports has appeared describing calcium homeostasis in normal and hypertensive pregnancy (tables 4.1.1 and 4.1.2), whereas others studied the effect of dietary calcium intake and oral calcium supplementation on blood pressure and the incidence of hypertensive disorders. In this review we will discuss calcium metabolism during normal pregnancy and the possible alterations associated with hypertensive disorders of pregnancy. The role of intracellular calcium will be discussed separately. Subsequently, we will discuss the current evidence for a relationship between calcium intake and hypertensive disorders of pregnancy from intervention studies and the putative mechanisms involved in the reduction of the incidence of hypertensive disorders by calcium supplementation.

Calcium homeostasis in normal pregnancies

A number of studies has been published that investigated calcium metabolism in normal pregnancy. Table 4.1.1 summarizes the main features of the studies. Ten studies reported on serum calcium and/or calcium hormone concentrations in all three trimesters of pregnancy[8,9,10,12,14,15,18,20,21,23] of which five were longitudinal[9,10,18,20,23]. Serum total calcium, which is either free or protein bound, is believed to decrease during pregnancy as the result of hemodilution and a consequent fall in albumin. In four studies this decrease was observed as early as the first trimester[10,14,18,21] whereas others reported this decline to occur in the second trimester[8,9,23] of pregnancy. However, in a number of studies serum total calcium levels were not different from non-pregnant controls during the first[8,9,20,23], the second[20,21] or the third[11,17,20,21] trimester of pregnancy. The serum ionized calcium concentration seems to be kept within narrow physiologic limits as the ionized calcium levels remained virtually unchanged throughout pregnancy[8,9,11,17,20,21,22,23], although a reduced level was reported in the second and third trimester of pregnancy compared to non-pregnant levels[10]. Additionally, Tan[24] and coworkers observed the ionized calcium level to decrease significantly from the first to the third trimester of pregnancy.

The most important calcium-regulating hormones are parathyroid hormone (PTH), PTH related protein (PTHrP), vitamin D metabolites, calcitonin (CT) and calcitonin gene-related peptide (CGRP). It has been postulated that a state of physiological hyperparathyroidism exists during pregnancy[25], as an adjustment to the expanding extracellular volume, the increased renal calcium excretion and the placental transfer of calcium to the fetus. However, PTH levels were decreased[9,20,21] or unchanged[10,18] in the first trimester, decreased[20,21,23] unchanged[18,19] or increased[10] in the second trimester and decreased[20,21,22,23] unchanged[11,13,14,15,16,18] or increased[9,10,19] in the third trimester of pregnancy compared with non-pregnant levels. In addition, Reddy et al[26] observed no increase in circulating PTH levels with advancing gestational age, although Rasmussen[27] found the PTH level to rise significantly from 18 to 36 weeks gestation. The controversy about serum PTH might be explained in part by the differences in assays used. However, the use of N-and C-terminal assays versus intact assays and cytochemical bioassays does no appear to account entirely for the discrepancies in the literature (table 4.1.1). Whether the PTH related substance PTHrP, which has a PTH like influence on bone and kidney, exert some effect on calcium metabolism during pregnancy is to date unknown. However, unlike PTH, whose synthesis is limited to the parathyroid glands PTHrP is screted by a number of organs, possibly including the placenta. Animal experiments showed that PTHrP plays an important role in the maintenance of fetal calcium homeostasis[28].

Data on serum 1,25-dihydroxyvitamin D show a significantly increased level in all three trimesters of pregnancy[11,12,13,15,20,23] compared with non-pregnant levels. Additionally, Kumar et al[12] observed a positive association between serum 1,25-dihydroxyvitamin D and the length of gestation, although Reddy et al[26] were not able to confirm this. It was suggested that a rise in 1,25-dihydroxyvitamin D is the primary event in the changes in calcium metabolism during pregnancy, yet Bouillon et al[29] considered it to be secondary to an increase in the concentration of vitamin D binding-protein as the free 1,25-dihydroxyvitamin D index remained normal up to 35 weeks of gestation. No direct information on free 1,25-dihydroxyvitamin D concentrations in pregnancy is available, but the observed increased

Table 4.1.1 Serum calcium and calcium regulating hormone levels in normal pregnant women compared with non-pregnant controls.

Reference		Pitkin '77[8]	Pitkin '79[9]	Drake '79[10]	Lund '79[11]	Kumar '79[12]	Steichen '80[13]	Conforti '80[14]	Whitehead '81[15]
Design (Longitudinal, Cross Sectional)		CS	L	L		CS		CS	CS
No. of subjects		137	30	9	15	31	19	58	42
Age of subjects (mean ± SD, range)			19-33	17-28		25 ± 4			19-36
1st trimester	Total Ca	NSD	NSD	decrease				decrease	
	Ca^{2+}	NSD	NSD	NSD					
	PTH		decrease[1]	NSD				NSD[1]	NSD[1]
	CT		NSD	NSD					increase
	1,25 ()$H)_2$ D					increase		NSD	increase
2nd trimester	Total Ca	decrease	decrease	decrease				decrease	
	Ca^{2+}	NSD	NSD	decrease					
	PTH		NSD[1]	increase[1]				increase[1]	NSD[1]
	CT			NSD				no diff	increase
	1,25 $(OH)_2$ D					increase			increase
3rd trimester	Total Ca	decrease	decrease	decrease	NSD			decrease	
	Ca^{2+}	NSD	NSD	decrease	NSD				
	PTH		increase[1]	increase[1]	NSD[1]		NSD[1]	NSD[1]	NSD[1]
	CT		NSD	NSD					increase
	1,25 $(OH)_2$ D				increase	increase	increase		increase

continued....

Reference		Gilette '82[16]	Richards '84[17]	Pederson '84[18]	Allgrove '85[19]	Gertner '86[20]	Davis '88[21]	Frolich '91[22]	Seki '91[23]
Design (Longitudinal, Cross Sectional)				L		L	CS		L
No. of subjects		10	16	18	10	16	81	10	20
Age of subjects (mean ± SD, range)		30 ± 1	24.2	26 (17-30)		27-43		29 (21-38)	22-34
1st trimester	Total Ca			decrease		NSD	decrease		NSD
	Ca^{2+}					NSD	NSD		NSD
	PTH			NSD[1,2]		decrease[1]	decrease[2]		decrease[2]
	CT			NSD					NSD
	1,25 $(OH)_2$ D					increase			increase
2nd trimester	Total Ca			decrease		NSD	NSD		decrease
	Ca^{2+}					NSD	NSD		NSD
	PTH			NSD[1,2]		decrease[1]	decrease[2]		decrease[2]
	CT			NSD					NSD
	1,25 $(OH)_2$ D					increase			increase
3rd trimester	Total Ca	decrease	NSD	decrease		NSD	NSD	decrease	decrease
	Ca^{2+}		NSD			NSD	NSD	NSD	NSD
	PTH	NSD[1]		NSD[1,2]	increase[2]	decrease[1]	decrease[2]	decrease[2]	decrease[2]
	CT			NSD					NSD
	1,25 $(OH)_2$ D					increase			increase

NSD=no significant difference, 1= N- or C-terminal assay; 2=intact assay

intestinal calcium absorption in pregnancy[30,31] might suggest a rise in both bound and free 1,25 dihydroxyvitamin D levels. Factors responsible for this increase, however, are unknown. Although PTH, estrogens, prolactin, growth hormone and human placental lactogen are able to increase the renal 1α-hydroxylation of 25-hydroxyvitamin D in various animal models, no correlations were observed between these hormones and 1,25-dihydroxyvitamin D levels in human pregnancy[26]. Another possibility might be an additional production of 1,25-dihydroxyvitamin D by the placenta[31,32]. To what extent the kidney and the placenta contribute to the increased synthesis of 1,25-dihydroxyvitamin D is still unknown.

Studies on CT secretion, which is regulated in direction opposite to that of PTH have yielded inconsistent results, with both unchanged[9,18,23] and increased[10,15] levels being observed (table 4.1.1). CGRP is derived from alternative processing of the calcitonin gene[33] and acts as a potent endothelium-independent vasodilator[34]. Whether CGRP concentration is influenced by calcium homeostasis during pregnancy is unknown.

Although parity has been related to an increase in bone mass and a decreased incidence of osteoporosis[35], the effect of pregnancy on the maternal bone mineral content is unclear. Lampe et al[35] observed a loss of trabecular bone between the early second trimester and one week post partum. In contrast, Christiansen et al[36] observed a constant bone mineral content throughout pregnancy. Similarly, Sowers et al[37] found no bone mineral density loss, when measurement taken before conception were compared with those post partum. In a longitudinal study, Seki et al[23] reported low serum osteocalcin, which is synthesized by the osteoblast and is proportional to the rate of bone formation, throughout pregnancy, indicating low bone turnover. Although, both PTH and 1,25-dihydroxyvitamin D stimulate the synthesis of calcitonin, it is unclear whether and how they may exert influence on bone mineral content during pregnancy.

Renal excretion of calcium increases during pregnancy[18,38,39] with maximum levels reached in the third trimester of pregnancy[18]. It was suggested that the hypercalciuria is mainly a consequence of the increased glomerular filtration rate. However, Gertner et al[20] observed no clear rise in fasting calcium excretion, whereas the urinary calcium excretion increased sharply after a calcium tolerance test, indicating that the hypercalciuria can be attributed to both an increased glomerular filtration rate and an increased intestinal calcium absorption. Additionally, it was postulated that normal pregnancy is also associated with a decreased tubular reabsorption[18], although Roelofsen et al[39] were not able to confirm this finding.

Calcium metabolism in hypertensive pregnancies

Studies comparing normal pregnancies to pregnancies complicated by hypertensive disorders are mainly confined to the third trimester of pregnancy (table 4.1.2). In general three forms of hypertensive disease can be distinguished. Elevations in blood pressure after the 20th week of gestation are called pregnancy-induced hypertension (PIH), or preeclampsia (PE) when hypertension is accompanied by proteinuria. When hypertension exist before pregnancy or is first noted before 20 weeks of gestation the diagnosis is chronic hypertension. Although the latter form of hypertension differs fundamentally from PIH/PE, chronic hypertension predisposes to the development of superimposed PE[3]. Results of studies of serum

calcium concentrations show no differences between women with pregnancy-induced hypertension (PIH), preeclampsia (PE) or chronic hypertension and normotensive women with respect to serum total calcium levels. Levels of serum ionized calcium, however, were either reduced in PIH[51], PE[40], and chronic hypertension[40] or were not different from normotensives[2,39,42,47,52]. A similar discrepancy can be observed regarding PTH and 1,25-dihydroxyvitamin D. Decreased PTH levels were observed in PE[47], PIH[51] and chronic hypertension[41], as well as an increased level in PE[40], whereas others reported no differences between PE[2,18,52] or chronic hypertensives[52] and levels in normotensive subjects. The 1,25-dihydroxyvitamin D was either decreased in PE[51] or not different in PE[42,47,51], PIH[42] and chronic hypertensives[52] compared with normotensives.

Some of the discrepancies may very well result from different criteria for hypertension and proteinuria in defining PIH and PE. Besides the international suggested guideline to denote PIH as a diastolic blood pressure ≥90 mmHg on two occasions and PE as PIH with concurrent proteinuria >0.5 g/24h, a rise in diastolic BP of ≥15 mmHg and a rise in systolic BP of ≥30 mmHg were also included in the definition of PIH in several studies[42,44,48,49,50,52]. Similarly, in some studies the definition of PE included a rise in serum uric acid >1 mg/dl and/or decreased platelet count[42], a rise in serum urate[44] and abnormal liver function tests and decreased platelet count[52].

A number of studies has reported decreased urinary calcium excretion in PE[18,40,42,43,44,45,47,48,49,50] when compared with normotensive pregnancies, although one study did not[52]. Similarly, PIH was associated with decreased urinary calcium excretion in two studies[43,45], which, however, could not be confirmed by Roelofsen et al[39]. When urinary calcium excretion in women with PE was compared with those with PIH a significant decrease was observed in the former[42,44,49]. Urinary calcium excretion represents a balance between glomerular filtration and tubular absorption. Several studies showed, in addition to decreased urinary calcium excretion, a decreased fractional calcium excretion[18,42,49,50] and an unchanged sodium excretion[40,42,47,50] (table 4.1.2) suggesting an increased distal tubular reabsorption of calcium in women with PE. Although Covi et al[45] observed a decreased sodium excretion in PIH and PE women, the sodium/calcium excretion ratio was more than doubled, indicating that the reduced sodium excretion does not entirely account for the reduction in urinary calcium excretion. Tubular reabsorption is affected by the calcium regulating hormones PTH and 1,25-dihydroxyvitamin D. However, unchanged levels of both hormones, even a decrease in PTH,
have been found in association with decreased urinary calcium excretion[18,42,47]. Whether alterations in calcium excretion precede the onset of PE is not clear, but the calcium-creatinine ratio at 24-34 weeks of gestation was found to be predictive of PE[53] in a longitudinal study. Sanchez-Ramos et al[48] also observed the relative risk of PE to exceed 9 for women with urinary calcium excretion below 195 mg/24h at 17 weeks of gestation, compared with those with calcium excretion levels above this value. At this time it is still unknown whether the relative hypocalciuria in PE may reflect a causal mechanism or should rather be viewed as a physiological response to other metabolic derangements.

Table 4.1.2 Calcium metabolism in hypertensive disorders of pregnancy.

Reference		Resnick '83[40]	Varner '83[41]	Pedersen '84[18]	Taufield '87[42]	Roelofsen '88[39]	Sowers '89[2]
No. group A: normal		8	?	18	10	46	34
No. group B: chronic hypertension			17		6		
No. group C: PIH					5	18	
No. group D: PE		9		15	19		13
Serum	total Ca			NSD A & D		NSD A & C	
	Ca^{2+}	↓ D vs A	↓ B vs A		NSD A,B,C & D	NSD A & C	NSD A & D
	PTH	↑ D vs A[1]	↓ B vs A[1]	NSD A & D[1,2]			NSD A & D[1]
	CT		↓ B vs A				
	$1,25(OH)_2$ D				NSD A,B,C & D		
Urine	Ca-excretion	↓ D vs A		↓ D vs A	↓ D vs A,B,C	NSD A & C	
	Na-excretion	NSD A & D			NSD A,B,C & D		
	Fe_{Ca}			↓ D vs A	↓ D vs A,B,C	NSD A & C	
	$Cr_{clearance}$	NSD A & D		↓ D vs A	↓ D vs A	NSD A & C	

continued....

Reference		Huikeshoven '90[43]	Hutcheson '90[44]	Covi '90[45]	Thong '91[46]	Frenkel '91[47]	Sanchez-Ramos '91[48]
No. group A: normal		24	84	30	20	11	91
No. group B: chronic hypertension			10			12	
No. group C: PIH		10	19	23	37		
No. group D: PE		7	21	7	20	14	8
Serum	total Ca	NSD A,B & D	NSD A,B,C & D				
	Ca^{2+}					NSD A,B & D	
	PTH					↓D vs A & B[2]	
	CT						
	$1,25(OH)_2$ D					NSD A,B & D	
Urine	Ca-excretion	↓ C & D vs A	↓ D vs A,B & C	↓ C + D vs A		↓ D vs A & B	↓ D vs A
	Na-excretion			↓ C + D vs A		NSD A,B & D	
	Fe_{Ca}						
	$Cr_{clearance}$					NSD A,B & D	NSD A & D

continued....

Table 4.1.2 (Cont'd.)

Reference		Sanchez-Ramos '91[49]	Seely '92[50]	Overloop '92[51]	August '92[52]
No. group A: normal		58	24	15	9
No. group B: chronic hypertension					9
No. group C: PIH		52		32	
No. group D: PE		33	12		9
Serum	total Ca				
	Ca^{2+}			↓ C vs A	NSD A,B & D
	PTH			↑ C vs A[1,2]	NSD A,B & D[1]
	CT			NSD A & C	
	$1,25(OH)_2$ D			NSD A& D	↓ D vs A & B
Urine	Ca-excretion	↓ D vs A & C	↓ D vs A		NSD A,B & D
	Na-excretion		NSD A & D		
	Fe_{Ca}	↓ D vs A & C	↓ D vs A		
	$Cr_{clearance}$	NSD A,C & D	NSD A & D		

PIH=pregnancy-induced hypertension, PE=preeclampsia, PTH=parathyroid hormone, CT=calcitonin. Fe_{Ca}=fractional calcium excretion, 1=N-or C terminal PTH, 2=intact PTH, NSD=no significant difference.

Intracellular calcium levels

Pregnancies complicated by PE are characterized by a general vasoconstriction[3] resulting in an increased peripheral vascular resistance. Intracellular calcium plays an important role in the initiation and maintenance of smooth muscle cell contraction and hence, vascular resistance. A small number of studies investigated basal intracellular calcium in platelets[54,55,56,57,58] and erythrocytes[2] as surrogates for vascular smooth muscle cells. Basal platelet intracellular calcium levels in normal pregnant women were not different from non-pregnant controls in the first and second trimester of pregnancy[56,58]. Similarly, no differences were observed in the third trimester[55,54,56], except by Kilby et al[58], who reported a significantly increased basal platelet intracellular calcium. In this study a significant correlation between blood pressure during pregnancy and intracellular calcium levels was found. In pregnancies complicated by PIH intracellular calcium in general was not different from normotensive women[55,57,58], although Sowers et al[2] observed an increased erythrocyte intracellular calcium in 13 women with PIH vs 34 normotensive women. In PE the platelet intracellular calcium levels were observed to be similar to normotensive pregnancies in the first, second[56] and third trimester[55,56,57] or increased in the third trimester of pregnancy[54,58]. These discrepancies across studies may partly result from differences in intracellular calcium measurements. However, changes in vascular resistance were not accompanied by comparable changes in platelet intracellular calcium[54,55,56,58]. Of course it is possible that changes in basal platelet intracellular calcium may not reflect vascular smooth muscle cell intracellular calcium. At present, abnormalities in cellular calcium handling in hypertensive disorders of pregnancy can not be excluded[59], but the biochemical mechanisms that might underlie an increased intracellular calcium in PIH and PE are still unclear.

Calcium intake and calcium supplementation

Over the past decade a vast number of population studies and intervention trials in non-pregnant subjects provided evidence to support the relationship between dietary calcium intake and blood pressure, although not all data are consistent[60,61]. An epidemiological link between dietary calcium intake and pregnancy-induced hypertension (PIH) was first postulated in 1980 by Belizan et al[62], who reported a low incidence of PIH in populations with high nutritional calcium intake. This hypothesis was supported by two randomized controlled trials, in which calcium supplementation significantly reduced blood pressure[63,64]. Additionally, Marcoux et al[65] observed the risk of PIH, but not of preeclampsia (PE) to reduce with increasing dietary calcium intake.

To date seven pregnancy-related supplementation trials have been published (table 4.1.3), investigating the effect of calcium supplementation on the incidence of PIH and PE. Although all trials compared results of supplemented with untreated women only five trials were double blind placebo controlled[64,68,69,70,71]. The sample size of the groups of women who received calcium supplementation varied considerably from 22 to 579. Additionally, the selection criteria of subjects for entry in to the study differed as well as the characteristics of the subjects. Five studies enroled healthy women only, although in two of these trials a specific test result ('rollover' test) was indicative for selection[64,68]. In three studies multiparous

women were excluded and in the remaining trials the proportion of multiparous women differed. These differences might affect not only the rate of PIH and PE, but may also influence the response to calcium supplementation. The latter may also be influenced by the dietary calcium intake, which varied from 300 to 1200 mg/day between trials. It could be anticipated that in women with low dietary calcium intake the effectiveness of calcium supplementation is larger. Table 4.1.3 shows that the gestational age at the start of supplementation and the daily supplemented dose of calcium differed among the studies. In one study[67] the calcium dose was combined with 1200 IU vitamin D. Although a dose-dependent effect as well as a relationship between length of supplementation and response might be expected, this has not been confirmed by the results of the studies. Another major problem in comparing the studies is the definition of PIH and PE. One study[64] did not provide a definition, in three studies PIH was defined as blood pressure ≥ 140/90 mmHg, whereas in the remaining three a rise in systolic blood pressure of ≥ 30 mmHg or a rise in diastolic blood pressure of ≥ 15 mmHg was also included in the definition of PIH. Similar discrepancies can observed in the way proteinuria was identified. In addition to the above mentioned differences in study design it is not clear in two studies whether reported data on PIH refer to PIH alone or include PE as well[64,66]. The results of the studies are presented in figure 4.1.1a and 4.1.1b. The incidence of PIH/PE was significantly reduced in three studies[68,69,71]. When PE was considered alone odds ratios were all less than 1.0, but in none of the studies statistical significance was reached (figure 4.1.1b). However, when odds ratios are pooled, the overall odds ratio estimates are 0.45 and 0.53, respectively (95% confidence interval 0.33-0.62 and 0.33-0.85), suggesting a favourable effect of calcium supplementation on the incidence of PIH and/or PE. In this area more data are needed to quantify the beneficial effect and to indicate which women might benefit most.

Possible mechanisms.

The mechanisms by which calcium supplementation may prevent pregnancy-induced hypertension and preeclampsia are currently unknown. Belizan et al[72] proposed parathyroid hormone (PTH) to play a major role in the observed reduced incidence of preeclampsia after calcium supplementation. They hypothesized that calcium supplementation reduces the PTH concentration, which in turn lowers the intracellular free calcium level, finally resulting in smooth muscle cell relaxation. The increased urinary calcium excretion observed in response to calcium supplementation[71,73,] might be attributed, in addition to an increased intestinal absorption, to a decreased tubular calcium reabsorption in response to lower PTH levels. However, PTH levels in calcium supplemented women were not significantly different from those who received a placebo[73]. The renin-angiotensin system has also been suggested to be involved in the protective effect of calcium supplementation on preeclampsia, as Repke et al[73] observed that low plasma renin activity and low serum calcium levels in pregnant women were good predictors of the blood pressure response to calcium supplementation. In addition, they observed a strong positive correlation between plasma renin activity and PTH. Although, plasma renin activity and calcium regulating hormones may interact in the development of preeclampsia[40] no data are available on the possible modifying effect of calcium intake on renin secretion.

Tabel 4.1.3 Summary of trials on the effect of calcium supplements on the occurrence of pregnancy-induced hypertension (PIH) and preeclampsia (PE).

Reference	Kawasaki '85[65]	Villar '87[64]	Marya '87[67]	Lopez-Jar. '89[68]	Lopez-Jar. '90[69]	Villar '90[70]	Belizan '91[71]
Design	Parallel	Parallel, DB, placebo	Parallel	Parallel DB, placebo	Parallel DB, placebo	Parallel DB, placebo	Parallel DB, placebo
Sample size	88	52	400	92	56	178	1167
nulliparas (%)	67	< 100	39	100	100	85	100
Age	18 - 39	18 - 30	20 - 35	≤ 25	19.4 ± 1.8	≤ 17	20 - 35
Gestational age at start (wks)	20	26	20 - 24	24	28 - 32	24	± 20
Dietary calcium intake (mg/day)	900	1000	500	300	300	1200	600
Calcium dose (mg/day)	156	1500	375 1200 IU vit D	2000	2000	2000	2000
no. PIH and PE/total							
Calcium	1/22	1/25		2/49	3/22	3/90	57/579
Placebo	14/42	3/27		12/43	24/34	8/88	86/588
no. PE/total							
Calcium			12/200		0/22	0/90	15/564
Placebo			18/200		8/26	3/88	23/588

DB=double blind.

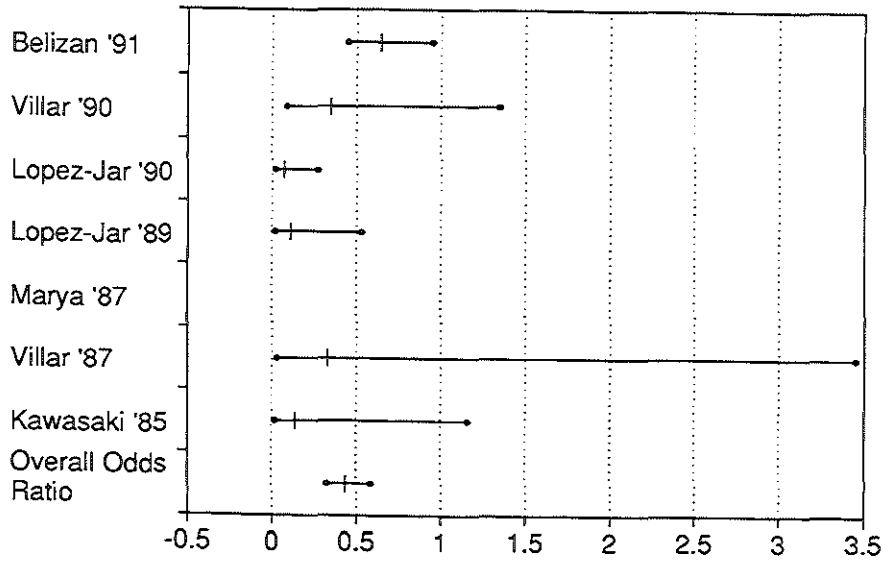

Figure 4.1.1a. Effect of calcium supplementation on pregnancy-induced hypertension and preeclampsia. Results are presented as odds ratios and 95% confidence intervals. The use of this strategy for data presentation may result in slight discrepancies between inferences in the original papers and those presented here.

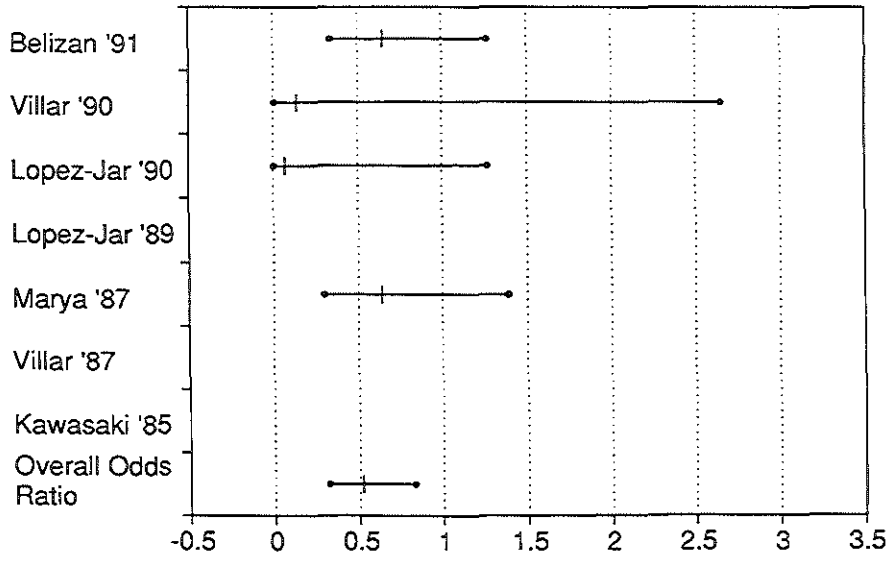

Figure 4.1.1b. Effect of calcium supplementation on preeclampsia.
Results are presented as odds ratios and 95% confidence intervals. The use of this strategy for data presentation may result in slight discrepancies between inferences in the original papers and those presented here.

Preeclampsia is associated with an exaggerated response to vasoactive hormones[5,54,56,57] and it could be anticipated that, in addition to a possible direct effect on smooth muscle cell contraction, calcium intake may also have indirect effects by modifying the responsiveness to or regulating the synthesis of a number of vasoactive agents, such as angiotensine II and endothelin (vasoconstrictor substances), and prostacyclin and endothelial-derived relaxing factor (vasorelaxing substances)[4]. Kawasaki et al[66] suggested that calcium supplementation may reduce the vascular sensitivity to vasopressor agents, as they observed a significant reduction in vascular sensitivity to angiotensin II after supplementation of 156 mg calcium. In addition, Lopez-Jaramillo et al[74], who found a relationship between extracellular calcium concentrations and the vascular synthesis of prostacyclin, postulated that calcium supplementation may enhance this synthesis[74].

At present, the mechanisms by which calcium supplements may interfere with vascular sensitivity have not been clarified as yet.

Recently, evidence has been provided that the parathyroid gland synthesizes a circulating hypertensive factor (PHF), which has been found in subject with essential hypertension[75,76] and which seems to be influenced by dietary calcium intake. However, no information is available about PHF and hypertensive disorders of pregnancy.

Interaction of calcium intake with intake of other nutrients

In identifying the relationship between calcium intake and blood pressure and the incidence of hypertensive disorders of pregnancy other nutritional factors may be of importance. In several studies, performed in non-pregnant subjects, the relationship between blood pressure and dietary calcium intake has been linked to other nutritional factors, including sodium, magnesium and potassium[77,78,79].

Although significant positive relations between blood pressure and sodium[80,81,82], inverse associations between blood pressure and potassium[78,83] and magnesium[84,85] have been observed, data are inconclusive and the role of specific nutrients in the development of hypertension is still unsettled. Data regarding the interaction between calcium intake and the intake of the mineral elements sodium, potassium and magnesium are sparse. A possible interaction between calcium and sodium intake was postulated by Resnick et al[86]. In view of their finding that subjects with high urinary sodium excretion tended to have the greatest decline in blood pressure after calcium supplementation they hypothesized that high sodium intake suppresses renin activity, resulting in an increased intracellular calcium. In addition, animal experiments show that the antihypertensive effects of dietary calcium may be in part sodium dependent[87]. However, McCarron et al[88] observed the greatest blood pressure reduction with calcium supplementation to occur amongst subjects with a low sodium intake. It was suggested that high sodium intake potentially increases urinary calcium loss, as a rise in sodium excretion is associated with an obligatory increase in calcium excretion[89]. On the other hand, no difference in blood pressure effect according to level of baseline sodium excretion has been reported[90].

Although potassium and magnesium are important factors in the control of vascular tone and reactivity, no data on a possible modifying effect of potassium and magnesium intake on the relationship between calcium intake and blood

pressure are available to date. However, it has been suggested that in pregnant animals[91] and non-pregnant subjects[92] a magnesium deficient diet may affect the intracellular calcium content and hence vascular tone. A decreased extracellular magnesium may decrease Na^+/K^+-ATPase activity allowing excess entry of calcium into the cell, finally leading to hypertension.

The association between calcium intake and blood pressure and hypertensive disorders has been described mainly for dairy products consumption rather than for total calcium intake. This is hardly surprising as dairy products account for approximately 80% of total calcium intake in westernized societies[93]. Since people consume milk rather than individual nutrients and, given the high degree of colinearity between the intake of calcium and magnesium in dairy products, it is difficult to isolate the effect of calcium on blood pressure and hypertensive disorders from magnesium and other minerals or even protein in dairy products. Despite the generally consistent finding of an inverse association between calcium intake and blood pressure and hypertensive disorders it can not be excluded that other dietary factors interact with or influence this association and further research is needed to disentangle the effect of nutrients and minerals present in dairy products. At present, however, calcium seems the most likely factor responsible for the observed association with blood pressure, findings that are supported by benificial effects of calcium supplementation in pregnant women.

Conclusions

This review presents an overview of the current state of knowledge of calcium metabolism in both normal pregnancies and pregnancies complicated by hypertensive disorders (i.e. pregnancy-induced hypertension, preeclampsia and chronic hypertension). Although pregnancy has been considered as a state of physiological hyperparathyroidism, recent studies are inclined to attribute the changes in calcium metabolism to a rise in 1,25-dihydroxyvitamin D rather than increased parathyroid gland activity. In pregnancies complicated by preeclampsia the urinary calcium excretion seems to be reduced compared to normal pregnancies and it has been postulated that the relative hypocalciuria precedes preeclampsia. At present, however, no unifying conclusion can be drawn due to inconsistency of the research findings, which is not entirely due to methodological differences. A large longitudinal study starting in the first trimester of pregnancy might provide more clarity.

Data regarding the involvement of intracellular calcium in the development of hypertensive disorders of pregnancy are inconsistent and no associations between intracellular calcium and vascular resistance have been observed. Although this might indicate that intracellular calcium levels are not directly related to the changes in vascular resistance as seen in PIH and PE, it does not allow to dismiss the possibility that abnormalities in cellular calcium handling might be involved.

Data from observational studies are suggestive of a relationship between dietary calcium intake and hypertensive disorders of pregnancy, but results of a number of clinical trials have provided varying results. Large methodological differences between these studies and differences in study populations make it difficult to compare and generalise results. However, despite these differences, most trials tend to point towards a beneficial effect of calcium supplementation.

In conclusion, there is sufficient circumstantial evidence that abnormalities in calcium metabolism may be involved in the etiology of both pregnancy-induced hypertension and preeclampsia to warrant further studies. Whether increased calcium intake and calcium supplementation are beneficial in the prevention and treatment of hypertensive disorders of pregnancy remains to be established.

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4.2

SERUM CALCIUM CONCENTRATIONS, DIETARY CALCIUM INTAKE, BLOOD PRESSURE AND UTERINE ARTERY FLOW VELOCITY WAVEFORMS IN PREGNANCY

Abstract

Calcium homeostasis in pregnancy has been related to peripheral vascular resistance on the basis of the importance of intracellular calcium in the regulation of vascular smooth muscle tone. Available data suggest that high dietary calcium intake during pregnancy has a positive effect on blood pressure and might decrease the incidence of hypertensive disorders of pregnancy. In this prospective study of 393 women of 35 years and older, serum total calcium, ionized calcium and dietary calcium intake in the first and late second trimester of pregnancy were examined in relation to blood pressure, uterine artery flow velocity waveforms and pregnancy outcome.

Systolic blood pressure was significantly associated with first trimester serum total calcium and ionized calcium (coefficient of linear regression (β)=21.7 mmHg/(mmol/l), 95% CI 11.4 to 31.9, adjusted for albumin and β=25.3 mmHg/(mmol/l), 95% CI 5.4 to 45.2, adjusted for body mass index).
Diastolic blood pressure was associated with ionized calcium in the first and the second trimester of pregnancy (β=17.7 mmHg/(mmol/l), 95% CI 3.8 to 31.6, adjusted for body mass index and β=16.4 mmHg/(mmol/l), 95% CI 1.4 to 31.5). No associations were observed between serum calcium levels and uterine artery pulsatility index (PI). Although absolute dietary calcium intake was not related to blood pressure levels or PI, an increase in dietary calcium intake between the first and the second trimester of pregnancy was associated with a decrease in diastolic blood pressure of 5.8 mmHg (95% CI -11.1 to -0.6).
Serum calcium levels and dietary calcium intake were not associated with pregnancy outcome.

The results of this study, conducted in a population of women with an average ample dietary calcium intake, are compatible with an effect of serum calcium levels and dietary calcium intake on blood pressure during pregnancy. The nature and clinical significance of these associations remains to be established.

Introduction

Pregnancy is hemodynamically characterized by an increased cardiac output and reduced peripheral vascular resistance due to a number of physiological changes relating morphological and biochemical mechanisms at the level of arterial resistance vessels. Although the relative importance of these mechanisms and their functional interrelationship is still largely unknown, disturbances in the physiological balance may result in increased peripheral vascular resistance as

seen in preeclampsia. It is apparent that vaso-active factors are involved in vascular resistance and, in part based on the role of intracellular free calcium in the regulation of vascular smooth muscle tone[1], increasing attention has focused on the possibility that preeclampsia is associated with alterations in calcium metabolism.

Calcium homeostasis in pregnancy has extensively been studied in both normal and hypertensive pregnancies[2,3,4]. Although virtually all metabolic studies show a decline in total serum calcium[5] during the course of normal as well as hypertensive pregnancy, controversy still exists as to the changes in serum ionized calcium and parathyroid hormone.

Additionally, several reports described an inverse association between dietary calcium intake and the incidence of hypertensive disorders during pregnancy[6,7] and a blood pressure lowering effect of calcium supplementation[8] in normotensive and hypertensive pregnant women has repeatedly been suggested.

The present study was conducted to compare longitudinal changes in blood pressure and Doppler uterine artery waveforms in relation to serum calcium levels and dietary calcium intake in the late first and late second trimester of pregnancy of women of advanced maternal age. Furthermore, the association between serum calcium concentrations and dietary calcium intake and the incidence of pregnancy complications was investigated.

Subjects and methods

Population

Subjects were recruited from patients attending the Prenatal Diagnosis Unit of the Academic Hospital Dijkzigt in Rotterdam for a late-first trimester chorionic villus sampling on the indication of advanced maternal age. The study was approved by the local Ethics Review Board and informed consent was obtained from each participant.

Criteria for eligibility included: diastolic blood pressure at recruitment below 85 mmHg, no history of cardiovascular disease or diabetes, a viable singleton pregnancy not beyond 11 weeks and maternal age 36 years or older at 20 weeks' gestation.

All women were recruited between 7 and 11 weeks of gestation and one follow-up visit was made between 23 and 27 weeks of gestation. At the initial visit fetal viability and gestational age were confirmed by ultrasound.

Measurements

Blood pressure was recorded in the right arm with a random zero sphygmomanometer; the reported values represent the mean of two readings taken at a one minute interval. Uterine artery flow velocity waveforms were recorded by means of a 4 MHz continuous wave Doppler ultrasound system (Doptek, Chichester, UK). The average of the left and right uterine arteries is presented by the mean pulsatility index[9] (PI). Blood pressure and uterine artery flow velocity waveforms were recorded with the woman in the semi-recumbent position. Venous blood samples were obtained for the determination of serum total calcium, ionized calcium and albumin, measured by standard laboratory methods. Laboratory analyses were performed simultaneously to exclude laboratory drift. Intake of calcium was estimated by a validated semi-quantitative food frequency

questionnaire[10], administered to 330 women at the initial visit and to 351 women at the follow-up visit; 296 women completed a questionnaire at both visits.

Data on pregnancy outcome and complications were followed-up by means of a questionnaire sent to referring obstetricians and midwives. The rates of hypertensive disorders of pregnancy (pregnancy-induced-hypertension (PIH) and preeclampsia), gestational diabetes, antepartum haemorrhage (bleeding from the genital tract after 28 weeks of gestation) and delivery by cesarean section were assessed. PIH was defined as diastolic blood pressure ≥90 mmHg or an increase of ≥20 mmHg on two separate occasions, preeclampsia was defined as PIH with proteinuria >500mg/24h or urine semi-quantitative dipstick testing of ++ or more. The rates of spontaneous abortion, genetic abortions, preterm delivery (<37 completed weeks of gestation), and small-for-gestational age (SGA, birth weight <10th centile for gestational age and sex) infants were computed as well. One intrauterine death was reported and one maternal death due to amniotic fluid embolism.

Data analysis

At baseline data of 393 women were available for analysis. In 20 pregnancies spontaneous abortion occurred, 10 were terminated because of chromosomal abnormalities and 11 women were lost to follow-up. This left 352 women for the follow-up visit. At this time one woman was hospitalized and missing data from this subject were included in the analysis as missing values.

The relationship between serum calcium concentrations, dietary calcium intake, blood pressure and PI at baseline and follow-up measurement was studied by multiple regression analysis. Secondly, the associations between change in serum calcium concentrations and dietary calcium intake and change in blood pressure levels and PI was examined. Thirdly, the risk for each of the outcome variables was estimated for increases in serum calcium concentrations and dietary calcium intake by means of logistic regression analysis. Results are presented with 95% confidence intervals and two-sided p-values when appropriate. In the analysis, a positive association was observed between baseline dietary calcium intake and the spontaneous abortion rate (relative risk = 2.8 /g/day, 95% CI=1.3 to 5.8). However, one of the women reported a calcium intake of 4.2 g/day (260% above the mean); after exclusion of this woman in the analysis the association was no longer present.

In the analyses performed, numbers do not all add up to the total number of women available due to slight differences in the number of missing values for the various parameters.

Results

Serum total calcium levels were strongly associated with albumin at both baseline and follow-up (coefficient of linear regression (β)=0.015 (mmol/l)/(g/dl), 95% confidence interval (CI) 0.011 to 0.019 and β=0.019 (mmol/l)/(g/dl), 95% CI 0.015 to 0.022). Similarly, was serum total calcium significantly associated with ionized calcium (β=1.08 (mmol/l)/(mmol/l), 95% CI 0.90 to 1.26 and β=1.01 (mmol/l)/(mmol/l), 95% CI 0.81 to 1.21, at baseline and follow-up, respectively). No associations were observed between both serum calcium levels and dietary calcium intake.

Serum total calcium, ionized calcium and dietary calcium intake were not associated with maternal age, gestational age, body mass index and parity, except for a positive association between baseline ionized calcium and body mass index (β=0.002 (mmol/l)/(kg/m^2), 95% CI 0.0004 to 0.004).

At baseline, systolic blood pressure was positively associated with the serum total calcium and ionized calcium concentration (β=21.7 mmHg/(mmol/l), 95% CI 11.4 to 31.9, after adjustment for albumin and β=25.3 mmHg/(mmol/l), 95% CI 5.4 to 45.2, after adjustment for body mass index). Diastolic blood pressure, however, was associated with ionized calcium only (β=17.7 mmHg/(mmol/l), 95% CI 3.8 to 31.6, after adjustment for body mass index). At follow-up, no associations existed between blood pressure levels and the serum total calcium concentration, whereas a positive association was observed between diastolic blood pressure and ionized calcium levels (β=16.4 mmHg/(mmol/l), 95% CI 1.4 to 31.5). No associations were found between both serum calcium levels and PI. Similarly, was dietary calcium intake not associated with PI or blood pressure levels.

The mean difference in gestational age between baseline and follow-up measurement was 15 weeks (range 13-19 weeks). In this period a significant fall in blood pressure levels and PI was observed. Also serum total and ionized calcium decreased significantly, whereas the average dietary calcium intake increased with 120 mg/day (table 4.2.1). The decrease in serum ionized calcium was significantly related to the change serum total calcium (β=0.1 (mmol/l)/(mmol/l), 95% CI 0.06 to 0.14, after adjustment for change in albumin). However, no clear relationship existed between change in dietary calcium intake and change in both serum calcium levels.

Table 4.2.1 Gestational age, serum calcium concentrations, dietary calcium intake, blood pressure levels, pulse rate and PI at baseline and follow-up.

	Baseline		Follow-up	
	n	mean (sd)	n	mean (sd)
Gestational age (weeks)	393	9.4 (0.9)	351	24.7 (0.8)
Total serum calcium (mmol/l)	391	2.27 (0.1)	351	2.20 (0.1)*
ionised calcium (mmol/l)	391	1.33 (0.05)	351	1.30 (0.04)*
albumin (g/l)	391	41.8 (2.0)	351	36.1 (2.0)*
calcium intake (g/day)	310	1.14 (0.6)	314	1.26 (0.6)*
Systolic BP (mmHg)	393	116 (11.4)	351	111 (10.2)*
Diastolic BP (mmHg)	393	68 (7.8)	351	65 (6.5)*
Pulse rate (beats/min)	375	73 (10.2)	351	80 (10.3)*
PI (0.01 units)	358	1.83 (0.4)	351	1.11 (0.2)*

* p<0.001 for change from baseline.

Between baseline and follow-up measurement diastolic blood pressure decreased significantly with 5.8 mmHg for every 1.0 mmol/l decrease in serum total calcium levels (95% CI -11.1 to -0.6). In addition a significant association was observed between the change in dietary calcium intake and diastolic blood pressure (β=-1.7 mmHg/(g increase/day), 95% CI -3.2 to -0.3).

No significant associations were found between serum calcium concentrations, dietary calcium intake and hypertensive disorders of pregnancy or any of the other adverse pregnancy outcomes.

Discussion

In this study we observed a significant association between serum calcium concentrations and blood pressure in the first and late second trimester of pregnancy. No associations were found between dietary calcium intake and blood pressure levels. However, an increase in dietary calcium intake from the first to the late second trimester of pregnancy was associated with a negative change in diasolic blood pressure. No relationship was observed between serum calcium levels or dietary calcium intake and pregnancy outcome.

Although comparison of food frequency questionnaire data with those derived from dietary records has indicated a reasonable level of validity[11], our measure of calcium intake was certainly not perfect. In the event that pregnancy-induced hypertension or preeclampsia during previous pregnancies might have caused women to adjust their diet during the course of the present pregnancy, the direction of any change would have been more likely to obscure relations between calcium intake, blood pressure and hypertensive disorders of pregnancy than to induce them. For this reason the strength of observed associations probably represents an underestimation of the true effects of calcium intake.

To our knowledge one other study has examined the relationship between serum calcium levels and blood pressure during pregnancy[2]. In contrast to our observations no association was observed between serum total calcium and blood pressure at any time during pregnancy in this study. In non-pregnant subjects, however, a significant positive association between systolic and/or diastolic blood pressure and serum total calcium has been reported[12,13,14]. No association appears to be present with ionized calcium[13,15], although Hvarfner et al[16] observed an inverse association between ionized calcium and mean blood pressure in healthy subjects. In addition, estimated dietary calcium intake was reported to be inversely related to diastolic blood pressure in men[17,18], but not in women[18]. In several studies in pregnant subjects an inverse relation between dietary calcium intake and blood pressure[19] has been described, as well as a reduced risk of pregnancy-induced hypertension with increasing calcium intake[20]. Calcium supplementation in pregnancy was found to lower blood pressure and reduce the incidence of hypertensive disorders of pregnancy[8,21,22]. The response of blood pressure to calcium supplementation appears dose-dependent as supplementation of 2g calcium/day may have a more beneficial effect than supplementation of 1g or 1.5g/day[8,19]. Two studies, both supplementing less than 0.5g/day, observed no beneficial effects of calcium supplementation on the incidence of hypertensive disorders[23,24]. Possibly this might be due to a threshold effect, below which the potential protective effect of adequate calcium intake decreases[25].

The mechanisms involved in a potential hypotensive effect of dietary calcium during pregnancy are poorly understood. Although no associations have been found between serum calcium levels and dietary calcium intake[26,22] mechanisms relating parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 may be involved. Unfortunately we have not determined PTH and vitamin D metabolites in the present study.

In summary, the findings of this prospective study suggest that serum calcium levels are associated with blood pressure during pregnancy. Although absolute calcium intake was not related to blood pressure, an increase in calcium intake seems to exert a positive effect on blood pressure. Whether serum calcium levels and dietary calcium intake are related to the occurence of hypertensive disorders of pregnancy remains to proven. Nevertheless, research on the role of calcium metabolism in blood pressure regulation during pregnancy may lead to a better understanding of the mechanisms behind hypertensive disorders of pregnancy.

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4.3

SERUM LIPIDS IN EARLY PREGNANCY AND RISK OF PREECLAMPSIA

Abstract

In a prospective cohort study of 393 women aged 35 years and older the association of serum total and HDL cholesterol with blood pressure, pulsatility index and pregnancy outcome was assessed during the first and late second trimester of pregnancy. First trimester serum total cholesterol was significantly associated with the risk of preeclampsia, with the gestational age and body mass index adjusted relative risk exceeding 5 for women with serum total cholesterol levels above 6.0 mmol/l when compared with women with a cholesterol level below 5.0 mmol/l. First trimester serum total cholesterol also showed a significant relationship with diastolic blood pressure (coefficient of linear regression=1.4 mmHg/(mmol/l), 95% CI 0.4 to 2.3), whereas a rise in HDL cholesterol of 1 mmol/l from the first to the late second trimester of pregnancy was associated with a positive change of 2.8 mmHg and 6.1 mmHg in diastolic and systolic blood pressure, respectively (95% CI 1.08 to 4.20 and 95% CI 3.97 to 8.22).

These data suggest a relationship between serum lipids in early pregnancy and the development of hypertensive disorders of pregnancy.

Introduction

During the course of normal pregnancy serum lipids and lipoprotein levels undergo serial changes and increases in serum triglycerides, cholesterol and to a lesser extent phospholipids can be observed[1,2,3]. The lipid changes are considered to reflect increased metabolic demands of the maternal organism. It is still unclear what causes these changes to occur, but the gestational hormones have been implicated.

Pregnant women of advanced maternal age appear to be at an increased risk of maternal morbidity compared with younger women, with pregnancy-induced hypertension and preeclampsia being the most common complications[4]. A decreased uterine circulation might play a part in the age-related increase[5,6] in hypertensive disorders of pregnancy, but the mechanism behind these complications is still largely unknown. Although data are sparse and not consistent, a more pronounced hyperlipidaemia has been reported in women with preeclampsia compared with healthy pregnant women[7,8,9].

In this study we examined whether total and HDL cholesterol levels in the first and late second trimester of pregnancy are associated with the development of pregnancy-related pathology in a cohort of healthy pregnant women of 36 years and older.

Subjects and methods

Population

393 pregnant women requesting late first trimester chorionic villus sampling on the indication of advanced maternal age were included in the study. Criteria for eligibility included: diastolic blood pressure at recruitment below 85 mmHg, no history of cardiovascular disease or diabetes, a viable singleton pregnancy not beyond 11 weeks and maternal age 36 years or older at 20 weeks' gestation.

All women were recruited between 7 and 11 weeks of gestation and one follow-up visit was made between 23 and 27 weeks of gestation. At the initial visit fetal viability and gestational age were confirmed by ultrasound.

Measurements

Serum total cholesterol and HDL cholesterol were measured by an automated enzymatic method using Boeringher-Mannheim CHOD-PAP reagent kit on serum samples stored at -20°C. HDL cholesterol was determined after precipitation of the remaining fractions. All measurements were carried out in the laboratory of the Department of Epidemiology & Biostatistics (Erasmus University Medical School, Rotterdam). Throughout the study accuracy and precision were within acceptable limits (WHO Regional Lipid Reference Centre, Prague). Blood pressure was recorded in the right arm with a random zero sphygmomanometer; the reported values represent the mean of two readings taken at a one minute interval. Uterine artery flow velocity waveforms were recorded by means of a 4 MHz continuous wave Doppler ultrasound system (Doptek, Chichester, UK). The average of the left and right uterine arteries, presented by the mean pulsatility index[10] (PI) was used in the analysis. Blood pressure and uterine artery flow velocity waveforms were measured with the woman in the semi-recumbent position. Height and weight, data on smoking habits and obstetric history were obtained at the initial visit. Body mass index (BMI) was calculated as weight divided by height square. Data on pregnancy outcome and complications were followed-up by means of a questionnaire sent to referring obstetricians and midwives. The rates of pregnancy-induced hypertension (PIH), preeclampsia and gestational diabetes were assessed. PIH was defined as diastolic blood pressure ≥90 mmHg or an increase of ≥20 mmHg on two separate occasions, preeclampsia was defined as PIH with proteinuria >500 mg/24h or urine semi-quantitative dipstick testing of ++ or more.

The rates of spontaneous abortion, genetic abortion, preterm delivery (<37 weeks of gestation) and small-for-gestational age (SGA, birth weight < 10th centile for gestational age and sex) were computed as well. One maternal death (due to amniotic fluid embolism) and one intrauterine death were reported.

Data analysis

Analyses were performed by multiple linear and logistic regression analysis. The results of the linear regression analysis are presented as coefficients of linear regression with 95% confidence limits. The risks of PIH and preeclamsia were also estimated in categories of total cholesterol and HDL cholesterol with the lowest category as reference. The odds ratio's as derived from logistic regression analysis with corresponding 95% confidence limits were used as an approximation of relative risk.

Serum lipid measurements at follow-up were missing for 42 women (20 spontaneous abortions, 10 induced abortions, 11 lost to follow-up, 1 hospitalisation), so

analysis of change in serum total cholesterol and HDL cholesterol could be performed in 351 women.

Results

At baseline, at an average gestational age of 9.5 weeks, serum total cholesterol was significantly associated with BMI (coefficient of linear regression (β)=0.05 (mmol/l)/(kg/m^2), 95% confidence interval (CI) 0.02 to 0.07), whereas HDL cholesterol showed a significant inverse association with maternal age (β=-0.03 (mmol/l)/year, 95% CI -0.05 to -0.01). Significant increases with advancing gestational age were observed in both serum total and HDL cholesterol at the baseline measurement (β=0.09 (mmol/l)/week, 95% CI 0.00 to 0.18 for total cholesterol and β=0.07 (mmol/l)/week, 95% CI 0.04 to 0.11 for HDL cholesterol). At the follow-up measurement (between 23 and 27 weeks of gestation) these associations were no longer significant.

Total serum cholesterol was significantly correlated with HDL cholesterol at both baseline and follow-up (correlation coefficient (r)=0.27, p<0.001 and r=0.13, p<0.01).

Except for a significant positive association between baseline total serum cholesterol and diastolic blood pressure (β=1.4 mmHg/(mmol/l), 95% CI 0.4 to 2.3, adjusted for gestational age and BMI) no relations were found between serum lipids and blood pressure levels or uterine artery pulsatility index (PI).

Pregnancy-induced hypertension (PIH) and preeclampsia were observed in 26 and 11 women, respectively. No associations were seen between baseline or follow-up serum lipids and the risk of PIH. The risk of preeclampsia, however, increased significantly with increasing levels of baseline total serum cholesterol concentrations (RR=2.23 per mmol/l, 95% CI 1.17 to 4.22, adjusted for gestational age and BMI). This association no longer reached statistical significance at follow-up (RR=1.49 per mmol/l, 95% CI 0.89 to 2.48). The relative risk of preeclampsia according to categories of baseline serum total cholesterol is presented in figure 4.3.1. No associations were observed between serum lipids at baseline or follow-up and the risk of spontaneous abortion (n=20) the development of gestational diabetes (n=17), preterm delivery (n=19) and delivery of a SGA infant (n=36).

During follow-up mean serum lipid levels rose significantly, whereas blood pressure levels and PI showed a significant decrease (table 4.3.1). The change in total serum cholesterol was associated with parity; for each additional previous pregnancy the increase in serum total cholesterol was 0.11 mmol/l (95% CI=0.02 to 0.19, adjusted for gestational age). An inverse association existed with baseline BMI (β=-0.05 (mmol/l)/(kg/m^2), 95% CI=-0.07 to -0.02, adjusted for gestational age and parity). In addition a change in both systolic and diastolic blood pressure between baseline and follow-up was positively associated with an increase in HDL-cholesterol (β=6.10 mmHg/(mmol/l), 95 CI 3.97 to 8.22 and β=2.84 mmHg/(mmol/l), 95% CI 1.08 to 4.20, adjusted for gestational age; figure 4.3.2). Increases in total serum cholesterol and HDL cholesterol were not related to the development of preeclampsia or any of the other adverse pregnancy outcomes.

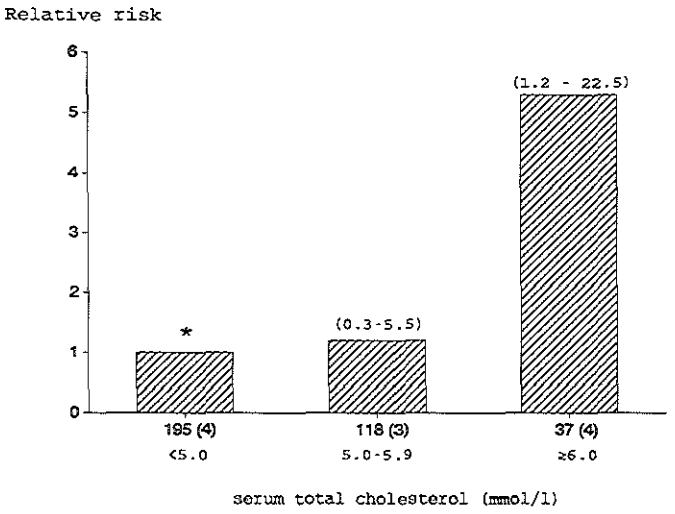

Figure 4.3.1 Relative risk of preeclampsia by categories of baseline serum total cholesterol. Relative risks are adjusted for gestational age and BMI. 95% confidence intervals are given on top of the bars. Numbers of women in the categories are given with numbers of women who developed preeclamsia in parentheses. * reference category.

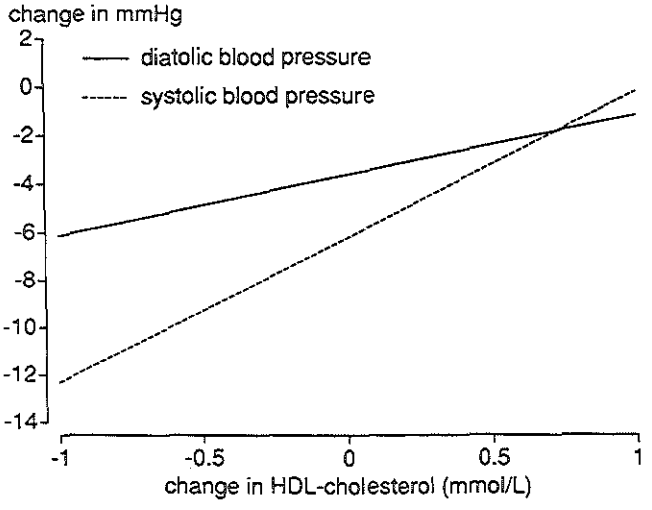

Figure 4.3.2. Change in systolic and diastolic blood pressure between the first and late second trimester by level of change in HDL cholesterol.

Table 4.3.1 Gestational age, serum cholesterol, blood pressure levels and PI at baseline and follow-up.

	Baseline		Follow-up	
	mean	(sd)	mean	(sd)
Gestational age (weeks)	9.4	(0.9)	24.7	(0.8)
Total serum cholesterol (mmol/l)	4.94	(0.8)	6.75	(1.1)*
HDL cholesterol (mmol/l)	1.69	(0.4)	1.97	(0.5)*
Systolic BP (mmHg)	116	(11.4)	111	(10.2)*
Diastolic BP (mmHg)	68	(7.8)	65	(6.5)*
PI (0.01 units)	1.83	(0.4)	1.11	(0.2)*

* $p<0.001$ for change from baseline.

Discussion

In this study among pregnant women of 35 years and older, a positive association was observed between first trimester serum total cholesterol level and the chance of developing preeclampsia. The relative risk of preeclampsia exceeded 5 for women with a cholesterol level of 6.0 mmol/l or higher at baseline compared with women with a cholesterol below 5.0 mmol/l, after adjustment for gestational age and QI. In addition, we observed that first trimester serum total cholesterol was positively related to diastolic blood pressure and a rise in HDL-cholesterol between the first and late second trimester of pregnancy was associated with an increase in both systolic and diastolic blood pressure.

Our data regarding increasing serum lipids during the first and second trimester of pregnancy are in concordance with other studies[2,3,9,11,12]. Hypercholesterolaemia is considered atherogenic and it increases the risk of coronairy heart disease[13]. This atherogenicity is mainly caused by the LDL cholesterol fraction whereas HDL cholesterol is considered antiatherogenic[13]. During pregnancy, however, both the LDL and HDL fractions have been reported to be elevated[2,12]. Although we observed an increase in HDL cholesterol from the first to the late second trimester of pregnancy the increase in total cholesterol observed in the same period might be caused by a high proportion of the total cholesterol that is carried by the LDL cholesterol fraction rather than the HDL cholesterol fraction, as the degree of correlation between total cholesterol and HDL cholesterol decreased between the two measurements. The hyperlipidaemia of pregnancy may originate from changes in the gestational hormones[14]. They exert their influence on HDL and LDL cholesterol levels by decreasing (estrogens) and increasing (progesterone) the hepatic lipase activity[14]. A decrease in hepatic lipase activity results in a similar accumulation of LDL and HDL cholesterol. This might indicate that the observed change in systolic and diastolic blood pressure between the two measurements is associated with LDL rather than HDL cholesterol. Possibly could the positive association of first trimester total cholesterol with diastolic blood pressure be attributed to the cholesterol carried by the LDL fraction as well. Unfortunately we have not determined LDL cholesterol in this

study.

Elevated levels of serum lipoproteins were reported in women with preeclampsia[7,8], but not in women with PIH[9]. These findings relate to the third trimester and at present no data are available about the first and second trimester of pregnancy. In the present study increasing total cholesterol levels at the first trimester had a significant effect on the risk of preeclampsia. The level of total cholesterol did not affect the risk of PIH. Possibly this might be related to the less severe character of PIH compared with preeclampsia, which is a generalized process that affects several organs including the liver and kidneys. In contrast to the effect of first trimester total cholesterol a contribution at the late second trimester to the risk of preeclampsia was not clearly seen. This might suggest that first trimester total cholesterol, which probably reflects in part pre-pregnancy levels, is a more important determinant of preeclampsia than the changes in total cholesterol that occur during pregnancy. Nevertheless, our findings reveal that total cholesterol and HDL cholesterol are associated with blood pressure, blood pressure changes and the development of preeclampsia.

To establish the importance of an elevated serum cholesterol in early pregnancy as a risk factor for the development of preeclampsia, understanding of the mechanisms involved in lipid en lipoprotein regulation in pregnant women is necessary.

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CHAPTER 5

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

In recent years a trend to delay the age of first childbearing until the mid-thirties has become apparent. Studies on the risk of maternal and fetal morbidity with advancing maternal age have shown inconclusive results. However, little doubt exists regarding an increased spontaneous abortion rate and increased rates of hypertensive disorders of pregnancy and diabetes with advancing age. This increase has been attributed to a decreased uteroplacental circulation. Doppler ultrasound allows non-invasive assessment of the uteroplacental circulation and qualitative information on the vessel under study can be derived from spectral analysis of the blood velocities thus obtained. In this thesis the pulsatility index (PI) was used as a measure of uterine artery blood velocity, providing indirect information on uteroplacental vascular resistance.

Data obtained from 393 pregnant women of 35 years and older followed prospectively during pregnancy were used to study the following primary research questions: 1) Do uterine artery flow velocity waveforms change during the first and second trimester of pregnancy? If so, are these changes associated with maternal age? 2) Are uterine artery flow velocity waveforms in the first and second trimester of pregnancy related to pregnancy outcome.

In the first and second trimester of pregnancy a decline in PI was observed, whereas in the late second trimester uterine artery PI remained virtually constant. These findings are in accordance with the characteristic decline of uteroplacental vascular resistance occurring in early pregnancy as a result of trophoblast invasion, which lasts until approximately 20 weeks of gestation. No maternal age dependent rise in uterine artery PI was observed in the first and early second trimester of pregnancy, but a positive association between age and uterine artery PI existed in the late second trimester of pregnancy. This suggest that maternal age might exert some effect on uterine artery blood velocity, but the strong association of maternal age with the spontaneous abortion rate and the lack of association with pregnancy complications make a substantial influence of maternal age on uteroplacental circulation seem unlikely.

The risk of spontaneous abortion increased significantly from 1.6% at age 35/36 years to 12.3% at 40 years and older, but uterine artery PI in the first trimester of pregnancy was similar in women who aborted and those who did not. Despite reports of an absent first wave of trophoblastic invasion with a subsequent abnormal arterial response to placentation, this might suggest that the well-established increased abortion rate in older women is due to poor oocyte quality rather than diminished uteroplacental perfusion. On the other hand, it could also anticipated that the uteroplacental circulation in the first trimester of pregnancy is determined not only by the degree of trophoblast invasion, but by multiple factors.

Pregnancy complications such as preeclampsia and intrauterine growth

retardation are frequently associated with an absent or defective second wave of trophoblast invasion, resulting in high peripheral vascular resistance. The increased risk of hypertensive disorders of pregnancy, small-for-gestational-age infants and, in addition, gestational diabetes observed in our study in women with high uterine artery PI at 12 to 13 weeks of gestation suggests not only that these hemodynamic changes are already detectable in early pregnancy, but they may, as suggested above, in part also reflect more general circulatory phenomena. The lack of a relation between fore-mentioned pregnancy complications and uterine artery PI in the late second trimester of pregnancy provides additional evidence that peripheral vascular resistance during pregnancy is influenced by a heterogeneity of factors. This assumption is further supported by the finding that the risk of preterm delivery strongly relates to uterine artery PI in the late second trimester.

In order to evaluate the potential modifying effect of several factors on peripheral vascular resistance during pregnancy the two following secondary research questions were addressed in this thesis: 1) Are serum calcium levels and dietary calcium intake associated with PI, blood pressure and/or pregnancy outcome? 2) Are total and HDL cholesterol related to PI, blood pressure and/or pregnancy outcome.

In the present study no associations were observed between uterine artery PI and serum calcium levels, dietary calcium intake, total and HDL cholesterol. However, the findings of linear increases in blood pressure levels with increasing ionized calcium, serum total calcium and serum total cholesterol concentrations in the first and/or second trimester of pregnancy, as well as the observation that the magnitude of change in calcium intake between the first and second trimester of pregnancy is inversely associated with blood pressure change in this period, suggests that vascular resistance during pregnancy may be influenced to some extent by such factors.

Whereas the risk of pregnancy complications, particularly hypertensive disorders of pregnancy, was not related to serum calcium levels or calcium intake, the risk of preeclampsia increased substantially with first trimester serum total cholesterol.

Further research is needed to evaluate the importance of elevated serum cholesterol levels in early pregnancy as a risk factor in the development of hypertensive disorders and to elucidate the mechanisms involved.

SUMMARY

The studies presented in this thesis were conducted in a cohort of healthy pregnant women of 35 years and older, who presented themselves for late first trimester prenatal diagnosis. This provided the opportunity to collect data on the uteroplacental circulation, as well as various other characteristics in early pregnancy. The main objective of the work presented was to document uterine artery flow velocity waveforms in the first and second trimester of pregnancy and to relate them to pregnancy complications and maternal age (chapter 1).

Uteroplacental Doppler studies in both normal and complicated pregnancies are evaluated by a review of the published results. In the interpretation of these studies, it is important to take into account the large methodological differences across studies, which include differences in study design, study populations, insonation sites along the uteroplacental vascular tree, indices used to describe the flow velocity waveforms obtained and definitions of normal and abnormal flow velocity waveforms. Most studies performed in normal pregnancies report a major drop in flow velocity waveform indices, i.e., vascular resistance, in the first half of pregnancy, which is believed to coincide with morphological changes in the uteroplacental spiral arteries as the result of trophoblast invasion. Studies assessing the predictive ability of uteroplacental Doppler flow velocity waveforms have yielded a large range in predictive values for the various complications, partly due to differences in choice of study populations(chapter 2).

The association between uterine artery flow velocity waveforms and the risk of spontaneous abortion was examined in 393 women, of which 20 aborted spontaneously. As expected, maternal age showed a significant positive association with the spontaneous abortion rate, but PI values in women who aborted were not different from those with ongoing pregnancies. Although spontaneous abortion has been associated with a defective first wave of trophoblast invasion, this suggests that the age-related increase in spontaneous abortion is attributable to poor oocyte quality rather than decreased uterine circulation. Alternatively it could also be postulated that multiple factors determine uteroplacental hemodynamics (chapter 3.1).

The risk of hypertensive disorders of pregnancy, gestational diabetes and small-for-gestational-age infants increased with increasing first trimester uterine artery PI values. This suggest that uteroplacental circulation in women who develop these complications might already be impaired in the first trimester of pregnancy. However, uterine artery PI in the late second trimester, did not show a similar association with the fore-mentioned pregnancy complications. Although increased uterine PI values have been ascribed to defective or absent trophoblast invasion these findings might indicate that an impaired first trimester trophoblast invasion is not necessarily associated with reduced uteroplacental circulation in later pregnancy. The observation that the risk of preterm delivery increased with

increasing uterine artery PI at the late second trimester of pregnancy suggests that other mechanisms are involved in the regulation of uteroplacental vascular resistance as well (chapter 3.2).

In addition to the associations between uterine artery flow velocity waveforms as a measure of vascular resistance and pregnancy complications we investigated selected parameters of calcium metabolism and serum lipids, which may potentially affect or modify vascular resistance, in relation to uterine artery PI, blood pressure and pregnancy outcome (chapter 4). Based on the role of intracellular calcium in the determination of vascular smooth muscle tone and hence, vascular resistance, and the suggested beneficial effect of high dietary calcium intake on blood pressure and the incidence of hypertensive disorders of pregnancy, growing attention has been directed towards calcium metabolism in pregnancy. A review of the current state of knowledge of calcium metabolism in normal pregnancy and in pregnancies complicated by hypertensive disorders is given in chapter 4.1. Although pregnancy has been considered a state of physiological hyperparathyroidism, recent data do not support this concept and are more inclined to attribute changes in calcium metabolism during pregnancy to an increased 1,25-dihydroxyvitamin D. However, as for now, research findings in both normal and hypertensive pregnancies show conflicting results and no unifying conclusions can be drawn. Similarly, are data on the relation between intracellular calcium and vascular resistance inconsistent. The results of calcium intervention studies are not unanimously supportive of a beneficial effect of calcium on the incidence of hypertensive disorders of pregnancy. However, despite a number of methodological differences, a tendency towards a positive effect of calcium supplementation can be detected in most studies.

Serum total calcium, ionized calcium and dietary calcium intake were studied in the same group of women participating in the previous studies. A significant association was observed between serum total an ionized calcium levels and systolic blood pressure in the first trimester of pregnancy, whereas diastolic blood pressure showed a significant positive relation with ionized calcium in the first and late second trimester of pregnancy. No associations were observed between serum calcium levels and PI. Similarly was absolute dietary calcium intake not related to blood pressure levels or PI. An increase in dietary calcium intake between the first and second trimester was, however, associated with a positive effect on blood pressure change, i.e. a decrease. Although no effect of serum calcium levels or dietary calcium intake on the incidence of pregnancy complications (i.e. pregnancy-induced hypertension and preeclampsia) was observed, the results are compatible with a beneficial effect of serum calcium and dietary calcium intake on blood pressure during pregnancy (4.2). The significance and mechanisms of such an effect remain to be established.

In the final study, attention was focused upon serum total cholesterol and HDL cholesterol levels. The risk of preeclampsia increased significantly with increasing first trimester total cholesterol levels. In addition, a positive association between first trimester serum cholesterol and diastolic blood pressure was observed. HDL cholesterol showed no associations with blood pressure levels or pregnancy outcome. HDL cholesterol rose by an average of 0.28 mmol/l between the first and late second trimester of pregnancy and this rise was associated with a decrease in both systolic and diastolic blood pressure. These findings suggest a relationship between serum lipid levels in early pregnancy and the development of hypertensive disorders of pregnancy.

SAMENVATTING

De onderzoeken beschreven in dit proefschrift werden uitgevoerd in een cohort van gezonde zwangeren van 35 jaar en ouder, die in aanmerking kwamen voor prenatale diagnostiek in het eerste trimester van de zwangerschap. Dit bood de mogelijkheid om gegevens te verzamelen over ondermeer de uteroplacentaire circulatie in de vroege zwangerschap. Het voornaamste doel van de onderzoeken was het vastleggen van bloedstroomsnelheidscurven van de arteria uterina in het eerste en tweede trimester van de zwangerschap en deze te relateren aan zwangerschaps complicaties en maternale leeftijd (hoofdstuk 1).

Uteroplacentaire Doppler studies in zowel ongecompliceerde als gecompliceerde zwangerschappen worden besproken aan de hand van de gepubliceerde resultaten. Bij de interpretatie is het belangrijk rekening te houden met de methodologische verschillen tussen de studies, zoals in onderzoeksopzet, de keuze van de studie populatie, de plaats bepaling van het te bestuderen uteroplacentaire bloedvat, de index gebruikt om de bloedstroomsnelheidcurve te beschrijven en de gebruikte definitie van normale en afwijkende bloedstroom-snelheidscurven. De meerderheid van de studies in de normale zwangerschap beschrijven een sterke daling in de bloedstroom-snelheidscurve index in de eerste helft van de zwangerschap, welke samengaat met morfologische veranderingen in de uteroplacentaire spiraal arteriën ten gevolge van trofoblast ingroei. Studies met betrekking tot de bruikbaarheid van uteroplacentaire bloedstroom snelheidcurven in de predictie van zwangerschaps complicaties lieten grote verschillen zien in predictieve waarden voor de diverse complicaties (hoofdstuk 2).

Het verband tussen bloedstroomsnelheidscurven van de arteria uterina en het spontane abortus risico werd bestudeerd in 393 vrouwen, waarbij bij 20 vrouwen daadwerkelijk een spontane abortus optrad. Zoals verwacht, is er een positief verband tussen maternale leeftijd en het optreden van spontane abortus, maar de pulsatility index (PI) bij vrouwen met een miskraam was niet verschillend van die bij vrouwen met een ongestoorde zwangerschap. Ondanks de relatie tussen spontane abortus en een gestoorde eerste fase van de trofoblast ingroei, lijken deze bevindingen aan te geven dat de leeftijd afhankelijke stijging van het spontane abortus risico eerder toegeschreven kan worden aan een verminderde kwaliteit van de oocyt dan aan een verminderde uterine circulatie. Het is echter ook mogelijk dat de uteroplacentaire circulatie door multiple factoren bepaald wordt (hoofdstuk 3.1).

Het risico op hypertensieve aandoeningen tijdens de zwangerschap, zwangerschapsdiabetes en SGA kinderen nam toe met het toenemen van arteria uterina PI gemeten in het eerste trimester. Dit wijst er op dat de uteroplacentaire circulatie bij vrouwen die deze complicaties in een later stadium ontwikkelen mogelijk al gedurende het eerste trimester gestoord is. Echter, in het tweede trimester werd

geen verband waargenomen tussen arteria uterina PI en voornoemde complicaties. Ondanks het feit dat een verhoogde PI van de arteria uterina toegeschreven wordt aan een gestoorde of afwezige tweede fase van de trofoblast invasie, wijzen deze bevindingen er op dat een gestoorde trofoblast invasie in het eerste trimester niet noodzakelijkerwijs met een verminderde circulatie van de uterus later in de zwangerschap gepaard gaat. De bevinding dat het risico om prematuur te bevallen toeneemt naarmate de PI gemeten in het tweede trimester toeneemt suggereert dat tevens andere mechanismen betrokken zijn in de regulatie van de uteroplacentaire vaatweerstand (hoofdstuk 3.2).
In aanvulling op het onderzoek naar het verband tussen arteria uterina bloedstroomsnelheidscurven, als maat voor vaatweerstand en zwangerschapscomplicaties, is tevens de relatie van een aantal geselecteerde parameters met betrekking tot calcium metabolisme en serum lipiden, welke mogelijk de vaatweerstand kunnen beïnvloeden, met arteria uterina PI, bloeddruk en zwangerschaps uitkomst onderzocht (hoofdstuk 4). Gebaseerd op de rol die intracellulair calcium speelt in de tonus van de vasculaire gladde spiercel en dus vaatweerstand, en het geopperde gunstig effect van een hoge calcium inname op bloeddruk en de incidentie van hypertensive aandoeningen tijdens de zwangerschap, wordt er steeds meer aandacht besteed aan het calcium metabolisme tijdens de zwangerschap. Hoofdstuk 4.1 geeft een overzicht van de huidige kennis met betrekking tot calcium metabolisme in zowel normale en hypertensieve zwangerschappen. Ofschoon zwangerschap gezien werd als een fysiologische hyperparathyroidie, wordt dit concept niet gesteund door resultaten van recente studies, die eerder geneigd zijn om de veranderingen die optreden in het calcium metabolisme tijdens de zwangerschap toe te wijzen aan een stijging van het 1,25-dihydroxyvitamine D. Echter, onderzoeks resultaten in beide, normale and hypertensieve zwangerschappen, tonen uiteenlopende bevindingen en er kunnen nog geen eenduidige conclusies aan verbonden worden. De resultaten van calcium interventie studies zijn niet eenduidig voor wat betreft een positief effect van calcium op de incidentie van hypertensieve aandoeningen tijdens de zwangerschap. Ondanks een aantal methodologische verschillen neigen de resultaten van het merendeel van de studies toch naar een positief effect van extra calcium.

Serum totaal calcium, geïoniseerd calcium en calcium inname werden bestudeerd in de zelfde groep vrouwen die aan de voorgaande studies deelnamen. In ons onderzoek bleek er een significant verband te bestaan tussen serum totaal- en geïoniseerd calcium en systolische bloeddruk in het eerste trimester. Diastolische bloeddruk liet een positieve relatie zien met geïoniseerd calcium in zowel het eerste als het tweede trimester. Serum calcium gehalte was niet geassocieerd met uterina PI. De absolute calcium inname liet ook geen verband zien met uterina PI of bloeddruk. Een gestegen inname van calcium tussen het eerste en tweede trimester was gerelateerd aan een sterkere bloeddruk verlaging in diezelfde periode. Serum calcium gehalte en calcium inname hadden geen effect op de incidentie van zwangerschaps complicaties (zwangerschapshypertensie en preeclampsie), wel zijn zij in overeenstemming met een positief effect op bloeddruk tijdens de zwangerschap (hoofdstuk 4.2). De waarde hiervan en de mechanismen hierachter dienen echter nog vast gesteld te worden.

In het laatste onderzoek staan serum totaal cholesterol en HDL-cholesterol centraal. Het risico op preeclampsie neemt significant toe met het toenemen van het serum totaal cholesterol bepaald in het eerste trimester. Tevens werd een verband tussen diastolische bloeddruk en serum totaal cholesterol waargenomen.

HDL cholesterol was niet gerelateerd aan bloeddruk of zwangerschaps uitkomst. HDL cholesterol steeg gemiddeld 0,28 mmol/l tussen het eerste en het tweede trimester en deze stijging hield verband met een daling in systolische en diastolische bloeddruk. Deze bevindingen wijzen op een relatie tussen serum lipiden in de vroege zwangerschap en het voorkomen van hypertensieve aandoeningen tijdens de zwangerschap.

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