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FACTORS ASSOCIATED WITH URINARY CATECHOLAMINE LEVELS IN MID-PREGNANCY

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Claudia Holzman
Major Professor's Signature

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FACTORS ASSOCIATED WITH URINARY CATECHOLAMINE LEVELS IN MID-PREGNANCY

By

Anjali Sapkal

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ABSTRACT

FACTORS ASSOCIATED WITH URINARY CATECHOLAMINE LEVELS IN MID-PREGNANCY

By

Anjali Sapkal

This study used data on mid-pregnancy urinary catecholamine (norepinephrine, epinephrine and dopamine) levels in 227 pregnant women who delivered at term and participated in the Pregnancy Outcome and Community Health (POUCH) study. For each catecholamine, day-to-day levels and waking and bedtime levels were moderately correlated (correlation coefficients of 0.18 to 0.60). Within each collection time (waking, before bed), norepinephrine and dopamine were highly correlated with each other (correlation coefficients of 0.75 to 0.90) but not with epinephrine. Catecholamine levels were lower in African-American women compared to white and other ethnicities and higher with advancing maternal age after adjusting for creatinine and smoking. Women who collected urine beyond 60 minutes post waking had lower epinephrine levels, but norepinephrine and dopamine levels were not significantly affected by this delay. No association was found between time of urine collection (waking and bedtime), work/non-work day and urinary catecholamine levels. In all the analyses, gestational age at enrollment was not significantly associated with any of the catecholamines levels.

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LIST OF ABBREVIATIONS

U.S.A.....United States of America
PTD..... Preterm Delivery
AFAM.....African-American
CRH.....Corticotrophin-Releasing Hormone
IL-1.....Interleukin-1
IL-6.....Interleukin-6
PSS..... Perceived Stress Scale
GA.....Gestational Age
SMS.....Sympathetic-adrenal Medullary System
HPA.....Hypothalamic-Pituitary Adrenocortical Axis
E.....Epinephrine
NE.....Norepinephrine
DA..... Dopamine
VMA..... Vanillylmandelic Acid
SES.....Socio-Economic Status
BMI.....Body Mass Index
PS.....Perceived Stress
PTSD.....Post-Traumatic Stress Disorder
LOD..... Limit of Detection
PMS.....Premenstrual Syndrome
PMM.....Premenstrual Magnification
MVA..... Motor Vehicle Accidents
MDD.....Major Depressive Disorder
BP.....Bipolar manic
PS.....Paranoid Schizophrenia
US.....Undifferentiated Schizophrenia
OAD.....Over-anxious Disorder
CR-PTSD.....Combat-related PTSD
Exp.....Experiment
IHV.....Intra-hepatic Vein

PCI.....Placental Cord Insertion

BDI.....Beck Depression Inventory

POUCH.....Pregnancy Outcomes and Community Health

MSAFP.....Maternal Serum Alpha-fetoprotein

ABPM.....Ambulatory Blood Pressure Monitor

LMP.....Last Menstrual Period

HPLC.....High Performance Liquid Chromatography

CV.....Coefficients of variations

GARS.....Global Assessment of Recent Stress Scale

L.....limit of detection

GLM.....General Linear Models

INTRODUCTION

Preterm Delivery: A Public Health Problem

World Health Organization defines preterm delivery as delivery before 259 days of gestation (i.e. before 37 completed weeks' gestation) (1). In the United States of America (U.S.A), preterm delivery (PTD) is the leading cause of perinatal mortality and morbidity. Despite a small decline from 1999 to 2000, the rate of PTD is higher now than in 1981, having risen steadily for almost two decades.

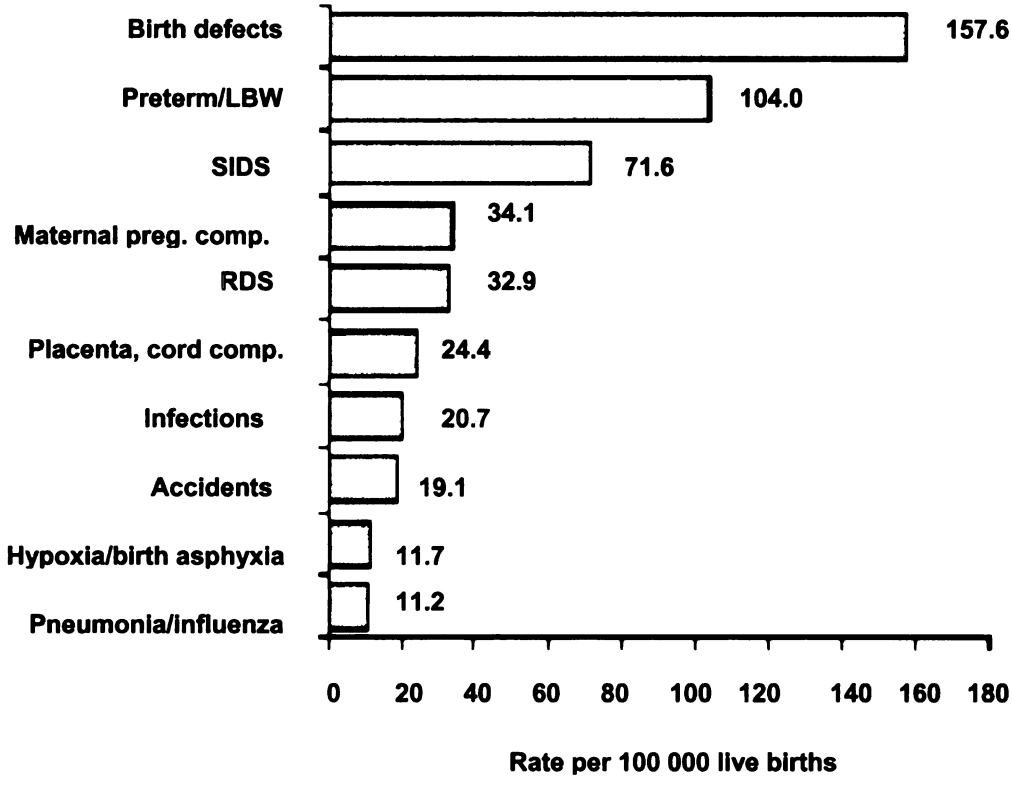

Figure 1: Leading causes of infant mortality (death during the first year of life) in the US among infants born in 1998
Source: National Center for health statistics, final natality data.

Figure 1 shows that preterm birth along with low birth weight is the second leading cause of infant mortality in the US at the rate of 104 per 100,000 live births.

For still unknown reasons, African-American (AFAM) women continue to have higher rates of PTD than the white women. AFAM women have a PTD rate of 16.3% whereas white women have a PTD rate of 8.1% chance (2) and this twofold risk disparity has existed for more than 50 years.

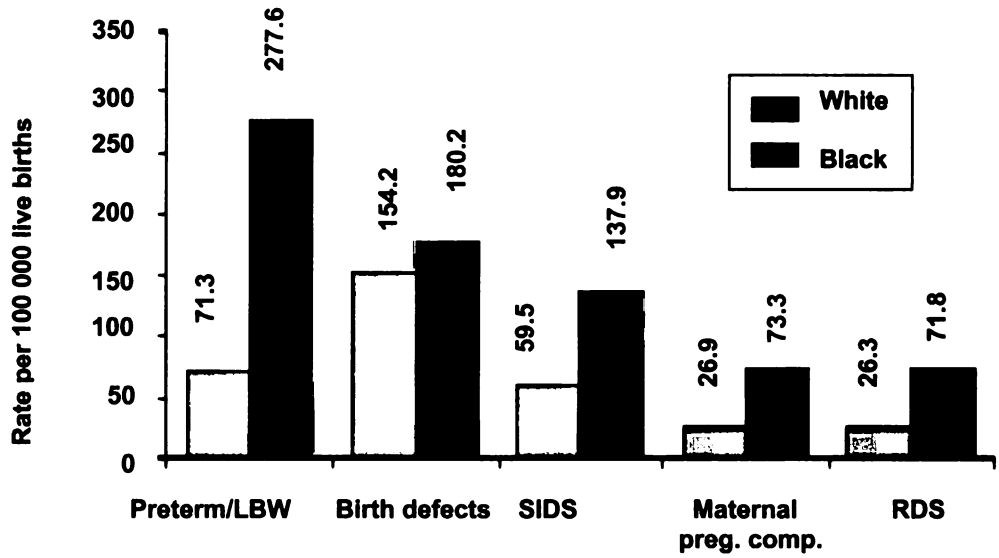

Figure 2: Leading cause-specific infant mortality rates by maternal ethnicity in the US in 1998
Source: National Center for health statistics, final natality data.

Figure 2 makes clearer the impact of PTD on AFAM infants. The AFAM infant mortality rate due to prematurity is 277.6 per 100,000 live births, which is substantially higher than congenital malformations, the leading cause of infant mortality in white babies.

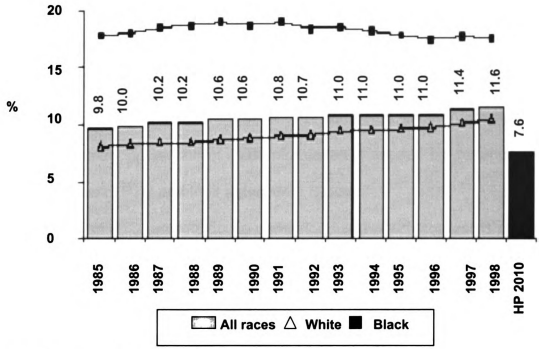

Figure 3: Preterm birth rate in the US over the period 1985-98
Source: National Center for health statistics, final natality data.

As seen in figure 3, the racial disparity has decreased because of an increase in PTD among white women and a small decrease in the PTD rate among African-American women. The figure also shows the goal for the U.S.A set by Healthy People 2010 as 7.6%.

PTD is associated with substantial emotional trauma to the family. At the community level it adds to the economic costs due to short and long term sequelae of PTD. Short term sequelae such as high mortality and morbidity and long term neurodevelopmental disabilities such as cerebral palsy, mental retardation and learning disabilities are higher among premature infants (3-5). Although improvement in gestational-age-related survival of preterm infants has occurred as a result of the use of antenatal corticosteroids, neonatal surfactant therapy and regionalization of perinatal care, there has been no reduction in the

incidence of preterm birth (6). Until now efforts to decrease PTD have mainly focused on secondary prevention such as use of tocolytic agents and early detection of labor. In spite of vast improvements in the field of medicine, the U.S.A continues to have a high rate of PTD as compared to other industrialized nations. For primary prevention of PTD, information is needed on the underlying biological causes of PTD and their antecedent factors.

The etiology of PTD remains poorly understood and when progress is made in this regard, maybe a meaningful reduction in the incidence of PTD is likely. Research into the causes and possible interventions to prevent PTD has important public health implications.

PTD is thought to have multiple pathways involving several factors. Some of the risk factors that have been explored include demographic characteristics such as ethnicity and socioeconomic status (7, 8) and biologic risk factors such as infection (9-11). During the past few decades, interest has been expressed in the potential etiologic role of psychosocial factors, including stress with PTD.

Preterm Delivery and Stress

Stress may lead to PTD, directly or indirectly. One direct pathway is through increases in maternal cortisol that stimulate increases in placental production of corticotrophin-releasing hormone (CRH), which leads to increased uterine contractility. Placental CRH production increases exponentially in the month preceding labor. CRH might lead to parturition either by stimulating the fetal pituitary-adrenal axis and production of fetal cortisol or by increasing prostaglandin production in the placenta and potentiating action of oxytocin. High

levels of perceived stress have been shown to be associated with increased CRH production, which might lead to PTD (12). High levels of stress produce elevated levels of catecholamines, resulting in vasoconstriction and subsequently oxygen and calorie reduction to the fetus. There is also some evidence that peaks of epinephrine precipitate labor and delivery (13-15). Stress can lead to an increase in cytokines (such as IL-1 and IL-6), which may then cause PTD or increase susceptibility to infection, thereby increasing the risk of PTD. Indirectly stress can lead to PTD by inducing risk behaviors such as smoking, alcohol, and drug use or influence the patterns of sexual activity (16, 17).

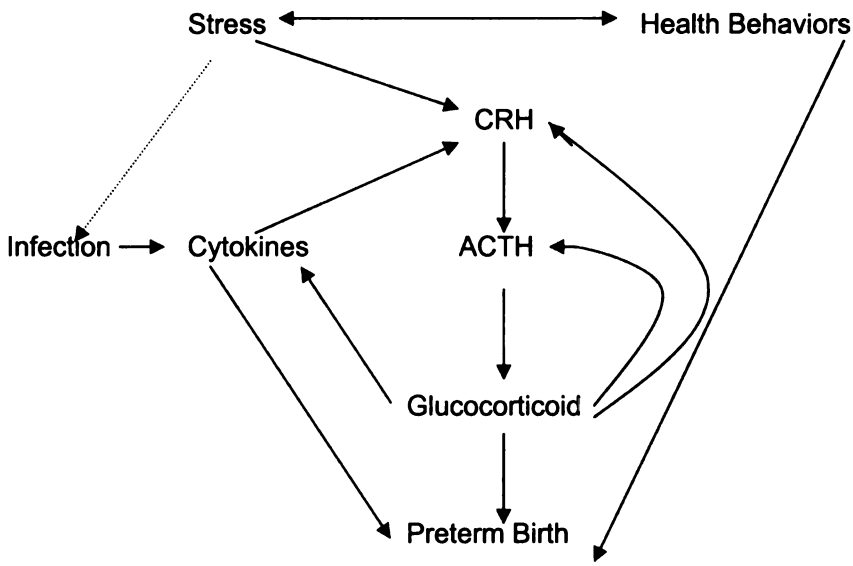

Figure 4: Schematic presentation of pathway of stress leading to preterm delivery
Adapted from Gennaro 2003 (17)

There is increasing evidence that stress might be associated with PTD (12, 13, 18-30) though studies have produced mixed results (31-36) (See Table 1 and 2).

Studies have been done in different countries such as USA, Australia, and Denmark and in different populations such as low socioeconomic class, African-Americans and nulliparous women.

Associations between stress and PTD have produced odds ratios ranging between 1.16 (26) to 3.39 (30). Measurement of perceived stress at 24 and 32 weeks was predictive of as much as 0.23 to 0.27 of the variance in gestational age (GA) at birth (21). Each unit increase in prenatal pregnancy anxiety was associated with a 3-day decrease in GA at birth (23). Pritchard CW et al (22) found the strongest association for the stress measure taken at 20 weeks where the odds ratio for those experiencing high levels of perceived difficulty was 2.86 (95% CI=1.05-7.76) for preterm birth. Dole et al (24) showed that women in the highest negative life events quartile had the highest risk of PTD (RR=1.8, 95% CI 1.2-2.7). Women having one or more highly stressful life events had a relative risk of PTD 1.76 as compared with those without stressful events with a 95% CI of 1.15-2.71 (25). The change in Perceived Stress Scale (PSS) score from 23-26 weeks of gestation to 31-35 weeks and gestational age at delivery had a correlation of $r=0.42$, $p<0.001$ (21). The greater the decrease in PSS scores, the longer was the gestational age. Lobel et al (13) found that stress significantly predicted PTD (standardized logistic regression coefficient=0.30, $p<0.03$). Copper et al (26) showed a significant association between stress and spontaneous PTD, an OR=1.16 ($p=0.003$). Odds ratio=2.12 was seen for 5+ life events in first two trimesters of pregnancy (19).

Rates of PTD increased with increasing levels of household strain within categories of Socio-economic Status (SES) (22). Greater the decrease in perceived stress score during the 2nd trimester, the longer was the gestational age (21). Copper et al (26) found a 16% increase in spontaneous PTD risk for every unit increase in the stress score. Berkowitz et al (19) provides evidence of significant linear trend between number of life events during the first 2 trimesters and the risk of PTD. Hobel et al (12) showed that maternal plasma levels of corticotrophin-releasing hormone are significantly elevated as early as 18 to 20 weeks' gestation in women who subsequently deliver preterm. In turn these changes in corticotrophin-releasing hormone are associated with maternal age and stress level at 18 to 20 weeks' gestation.

Studies have offered preliminary evidence that the effects of prenatal stress are highly specific for different birth outcomes (37). Personal resources (mastery, self-esteem, optimism) have been implicated in processes related to fetal growth but not to the timing of delivery, whereas stress defined as state anxiety was seen to be associated with length of gestation but not with fetal growth (29).

Studies have measured stress prospectively (12, 13, 20-30) early in pregnancy, hence time order is better established. Stress was measured in earlier part of pregnancy hence less likely to be due to complications experienced in pregnancy.

Rates of PTD are consistently highest among women with social disadvantages, African-Americans, poor, undernourished, single parents, lower education and income (18); while women with these characteristics are also

known to have high levels of stress. Maladaptive health behaviors such as smoking, substance abuse and poor weight gain during pregnancy are associated with increased rates of prematurity and it has been reported that these behaviors are more prevalent among stressed women.

New insights into the role of maternal stress on gestational length could lead to specific interventions to alleviate stress, improve mental health of pregnant women and thereby could decrease the risk for preterm birth.

Table 1: Literature Review on Stress and Preterm Delivery – Positive studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Wadhwa 1993/ Prospective/ N=90	Life events, perceived stress, anxiety, daily hassles	28 to 30th weeks (wks) of gestational age (GA)	PTD	Controlled for potential confounders	Small sample size	Association between prenatal pregnancy anxiety and PTD
Hobel 1999/ Prospective/ Nested c-c study/ N=524	Perceived stress, anxiety	18-20th, 28-30th, wks of GA	PTD	Matched controls to cases; subjects appraisal of stress used	Small number used for c-c study; measured stress experienced one month prior to the interview	Association between elevated CRH levels, prenatal stress and PTD
Hedegaard 1996/ Prospective/ N=5873	Life events, perceived stress, psycholog-ical distress	16th and 30th wk of GA	PTD	Stress measured twice; controlled for potential confounders	Includes both spontaneous and induced PTD	Association between events appraised as stressful and PTD

Table 1: Literature Review on Stress and Preterm Delivery – Positive studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Dole 2003/ Prospective/ N= 1962	Life events, perceived stress, pregnancy related anxiety, discrimina-tion	24-30 wks of GA	PTD	Prospective; external stress, perceived stress, buffer of stress all measured; covariates controlled; analyzed both spontaneous, medically induced PTD separately and combined	Women seeking late or no prenatal care could not be included	Association between PTD and negative life events
Pritchard 1994/ Prospective/ Nulliparous women excluded N=393	Household strain and stress	20th and 30th wk of GA	PTD	Repeated measures of exposure	Will miss the women who book late for prenatal care; single social role considered for measuring stress	Household strains are associated with an increased risk of PTD

Table 1: Literature Review on Stress and Preterm Delivery – Positive studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Lobel 1992/ Prospective/ Low socioeconomic status women; N=130	Perceived stress, life events, anxiety	14 to 30th wk of GA	PTD	Multiple indicators of stress used; repeated measurements over pregnancy; medical risk and parity controlled	Life events measured postnatally; external validity problematic; small number of cases	Earlier delivery was predicted by medical risk and by prenatal stress.
Ruiz 2001/ Prospective/ Predominantly Medicaid eligible; N=78	Perceived stress scale (PSS)	23-26 and 31-35 wks of GA	PTD	Repeated measures of cortisol, vaginal swabs, stress; part of data was prospective	Selection bias possible because all women came from 2 physician's private practice; out of total of 6 PTDs, for 5 part of data was collected after delivery; no confounders were controlled	Greater the decrease in PSS score, the longer was the GA.

Table 1: Literature Review on Stress and Preterm Delivery – Positive studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Copper 1996/ Prospective/ N=2593	Stress, anxiety, depress-ion, self-esteem, mastery	25 to 29 wks of GA	Spontan-eous PTD (<35 weeks)	Controlled for race, maternal age, marital status, insurance, education, tobacco, alcohol, drug use	Large number of assessments performed and one or more significant associations found could have occurred by chance	Stress significantly associated with spontaneous PTD
Berkowtiz 1983/ Case-control study/ Cases=166 Controls=299	Frequency of life events, attitude to pregnancy	Postpar-tum	Spontan-eous PTD	Adjusted for age, marital status, ses, gravidity	Retrospective data collection-"recall bias" possible; life events only during pregnancy measured	Higher number of life events and negative attitude to pregnancy associated with PTD
Nordentoft 1996/ Prospective/ N=2432	Life events, perceived stress	20^{th} week of GA	PTD	Controlled for covariates such as age, education, smoking, drinking	Lower limit of OR CI is 1.00, residual confounding cannot be ruled out.	Perceived stress was associated with PTD, OR= 1.14 (CI 1.00-1.29); attributable risk =3 %

Table 1: Literature Review on Stress and Preterm Delivery – Positive studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Bhagwanini 1997/ Prospective/ N=88	State and trait anxiety	8 to 28 weeks and repeated after minimum intervals of 6 weeks	PTD	Assessed anxiety levels 3 to 4 times during pregnancy	Recall bias; small sample size; restricted study population to nulliparous women	Significant association was observed in the 2nd trimester between low trait anxiety levels with PTD (p=0.019)
Rini 1999/ Prospective/ N=230	State and pregnancy anxiety	28-30 weeks (2 interviews 2 wks apart)	PTD	Controlled for potential confounders; simulta-neously considered adaptational resources and constraints operating both at contextual and individual levels	Inadequate power due to small sample size; GA used as continuous variable	Stress was significant predictor of PTD OR= 1.56

Table 1: Literature Review on Stress and Preterm Delivery – Positive studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Steer 1992/ Prospective/ N=323 adolescents; N=389 adults	Self reported depression	28 wks of GA	PTD	Controlled for potential confounders such as demographic, adverse health behaviors, obstetrical variables	External validity problems because sample consisted mainly of minority groups; no relation of depression with PTD seen in adolescents	For adults, at a cut off score of 21 for the BDI, the risk of PTD was 3.39 (95% CI 3.24-3.56)
Orr 2002/ Prospective/ N=1399	Depression	25% in 1st trimester and 75% at end of 2nd trimester	Sponta-neous PTD	Controlled for potential confounders	Restricted to African-American women	Association between depression and PTD, adjusted OR=1.96 (95% CI 1.04-3.72)

Table 2: Literature Review on Stress and Preterm Delivery – Negative studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Honnor 1994/ Prospective cohort/ High risk women for poor obstetric outcome; N=1061	Life events, appraisal	28 to 32 wks of GA	PTD	Stress measured earlier in pregnancy; events, appraisal both measured for stress; adjusted for potential confounders	All women were high risk for poor obstetric outcome, so likely that stress might be homogenously distributed in the cohort leading no difference between PTD and term pregnancies	No association between life events and PTD
Barbosa 2000/ prospective and retrospective data/ African American women N=472	Frequency of life events	1st prenatal visit, within 2 days after birth	PTD	Could examine indirect and direct relation between life events and PTD due to use of both prospective and retrospective data	Women with previous PTD excluded; considered frequency of life events only during pregnancy; recall bias; underreport data due to subject forgetting event or its impact	No association between total number of life events during pregnancy

Table 2: Literature Review on Stress and Preterm Delivery – Negative studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Norbeck 1989/ Prospective/ Low-income medically normal women/ N=208	Life events, anxiety	mid-pregnancy, late pregnancy	PTD	Only mid-pregnancy measure used as predictor variables; controlled for medical and demographic variables	Small number of cases-enough power?	No association between life events and PTD
Peacock/ Prospective/ Restricted to white women N=1513	Life events, anxiety, depression	At booking, 17, 28,36	PTD	Controlled for alcohol, smoking, caffeine, socioeconomic status, other demographic variables; analyses done separately for spontaneous and induced births	Life events measured at 36 wks so could not be used in final analysis; lack of significant results could be due to lack of power; problems with external validity due to restricted sample	No association with of anxiety or depression with PTD

Table 2: Literature Review on Stress and Preterm Delivery – Negative studies

Author/ Study/ Sample Size	Instrument	Stress measured time in gestation	Outcome	Strength	Weakness	Results
Pagel 1990/ Prospective/ N=100	Frequency of life events, state anxiety	21 to 36 wks	PTD	Controlled for potential demographic, biomedical, lifestyle variables	Measured only the frequency of events; summed the risks into an overall score; GA used as continuous variable	No association of life events with PTD
Stein A 1987/ Prospective/ Restricted to city of Oxford; N=483	Objective measure of stressful life events	6 weeks before expected date of delivery	PTD	Impact of short term and long term stressors assessed separately; collected data on stress from 12 months preceding interview	Selection Bias; sample had lower rate of PTD –did not have enough power to detect a difference; objective measure of stress and not subjects own appraisal; only smoking, low income, unemployment, parity controlled; the possibility that some PTD women might have been interviewed postpartum cannot be ruled out	No association of adverse life events and chronic difficulties with PTD

Weakness in Studies on PTD and Stress

1. Stress has been measured by various instruments such as life events, daily hassles, perceived stress and psychological distress (often measured by anxiety or depression). Life events counts and measures of objectively weighted life events are inadequate because they rely on the stimuli, without ascertaining whether they were appraised by the individual as stressful or elicited responses such as anxiety (31, 33, 34, 36). In some studies, questions were asked about stressful life events only during pregnancy, thus the effect of prior stressful events might have been missed (12, 19, 31).
2. Retrospective or cross-sectional collection of data on stress may face problems of recall bias, temporality and underreport of stress because subjects may not remember the occurrence or the impact of events over time. There are chances that women with adverse birth outcome may review their past in a more negative way (6, 19, 31).
3. Stress is typically measured at one time point during pregnancy(18, 19, 23, 24, 26, 27, 30, 32, 34, 36). It is not know if there is a critical point in pregnancy when experiencing acute stress is more detrimental to pregnancy outcomes than at other times or if chronic or acute stress leads to PTD.
4. Studies often fail to test for the direct as opposed to the indirect effects of stress (21, 36). For example, other risk factors for PTD, such as smoking, poor nutrition, drug use, previous PTD, and biomedical risks could be

added to models to test if stress is an antecedent to these potentially mediating factors and how much of the stress-PTD association is explained by these factors.

Challenges in Measuring Stress

Stress has numerous definitions and various measures by which it is assessed in literature (38, 39). One study defines stress as a psycho-physiological consequence of any event challenging an organism's capacity to cope (40). Stress has also been defined as a stimulus, as a response, and as the transaction between environmental stimuli and individual responses (37).

Three broad ways to measure stress described in literature are- (2) objectively as life events or experiences, (8) subjectively as individual's appraisal and (8) biologically due to the activation of physiological systems. Objectively stress can be assessed as a stimulus (stressor). Measurement of such a stressor is done commonly by adding the number of life events experienced by an individual or based on weighted scores to produce an overall life events index. Life events have been defined as environmental stressors that are threatening or harmful and which have the potential to impact adversely on health, whether it be physically or psychologically when it is associated with high state anxiety (40). High trait (personality-related) anxiety can further amplify the psychological response to life events stress. Life events measure has disadvantages of ignoring the subject's appraisal to the stressor and contributions of coping mechanisms.

Stress as a response can be psychological or physiological. Psychological stress can be measured by a variety of self-report (perceived stress) or interviewer ratings of anxiety, depression, distress, etc. Anxiety is considered as the psychological consequence of exposure to real or imagined stress (40). Perceived stress is a measure of individual's appraisal of the stressful situation, which is a more sensitive measure of stress than objective measure, since all individuals do not perceive life events as equally stressful. Physiological stress can be measured by various techniques such as cardiovascular response (heart rate, blood pressure, vagal tone), immunologically by antibody response, lymphocytic activity, interferon production and neuroendocrine response, which is based on activation of the sympathetic-adrenal medullary system (SMS) and the hypothalamic-pituitary adrenocortical axis (HPA). SMS and HPA are viewed as the primary indicators of a stress response and are known to release stress hormones such as cortisol and catecholamines.

Summary: Studies measuring stress in pregnancy conceptualize stress several different ways, which may explain inconsistencies in linking stress to pregnancy outcomes. As noted above there are numerous definitions of stress in literature and various instruments are available for measuring stress. Whether subjects are queried before or after the outcome of interest is important for obtaining the correct time frame of stress measurement. Retrospective collection of stress data may introduce recall bias since women with adverse pregnancy outcomes may view their past in a more negative manner. Measurements at single time in pregnancy might also be inadequate assessment of stress since

impact of some acute events might be missed. For studies on stress and pregnancy it is recommended to use repeated measurements, conduct prospective studies and use multidimensional approach to stress measurement because of its conceptual power and potentially greater predictive value (37).

Most of the studies on PTD and stress mainly focus on psychological measures of stress and fail to consider physiological measures of stress. Large-scale epidemiological studies have a great potential to examine the physiological links between stress and PTD, but many have failed to include potential physiological markers because it is unclear which ones to use and the assessment protocols are too burdensome. The difficulty in selecting potential stress biomarkers arises from diversity of markers examined (cortisol, catecholamine), the modes of assessing them (such as urine, saliva, plasma) and the times of sampling (24 hours, overnight, morning, evening). The burden of collecting urine throughout the day generally precludes such measures from being used in large-scale epidemiological studies. Several studies have shown largest differences in salivary cortisol to be at the beginning or end of the day and targeted sampling at those times could serve as a low-cost marker. There is also increasing evidence for the value of catecholamines as potential biomarkers of stress.

Catecholamines and Stress

It has been shown that urinary levels of catecholamines are associated with the activities, behaviors, attitudes, perceptions and stress responses in humans. The three catecholamines, Epinephrine (E), Norepinephrine (NE) and Dopamine

(DA) have been recognized as sensitive indicators of psychophysiological reactions.

The Biology of Catecholamines

Tyrosine is the precursor of all the catecholamines (41-45). Tyrosine hydroxylase forms dopa from tyrosine, which is decarboxylased into dopamine. Dopamine beta-hydroxylase converts dopamine into norepinephrine which is converted into epinephrine by N-methyl transferase (41-45). Epinephrine (E) is the principal hormone of the human adrenal medulla (approximately 90%) while norepinephrine (NE) is the neurohormone of the sympathetic nerves (41, 42, 45, 46). The distribution of catecholamine (E, NE and DA) within the sympatho-adrenal system is uneven. In the nerve cells of the sympathetic system, the ratio of DA to NE is about 50:50, E being totally lacking. In the adrenal medulla, on the other hand, there is about 5 times more E than NE, while DA represents only about 2 percent of the total amount of catecholamines present. Deviations in secretion of E may be expected with stimulation of adrenal medulla and changes in secretion of DA associated with stimulation of sympathetic system, while excretion of NE will occur in both instances (46, 47).

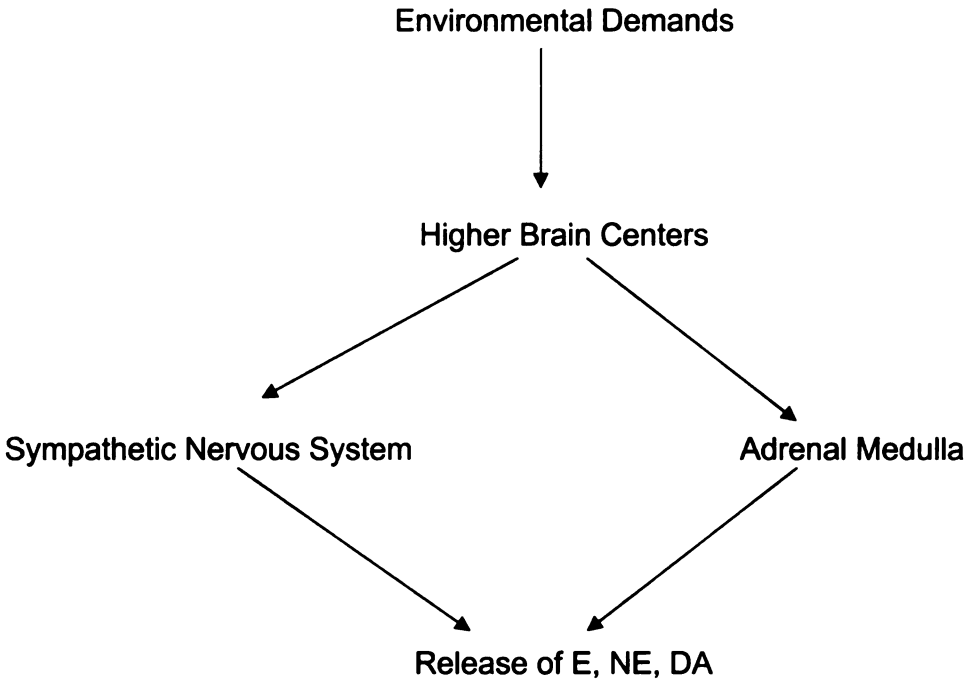

Figure 5: Schematic presentation of pathways from the brain to adrenal systems involved in stress
Adapted from Frankenhaeuser 1989 (48).

E has been recognized as the emergency ("flight and fight" reactions) hormone that prepares one to meet threatening situations by stimulating the heart, dilating the arterioles of the heart and skeletal muscles, mobilizing glucose and demobilizing the gut. NE has a major role in cardiovascular homeostasis; it has a general vasoconstrictor action, with the exception of dilation of the coronary vessels and raises both systolic and diastolic pressures (49).

The adrenal medulla directly discharges E into the blood and hence blood levels of E reliably reflect changes in adrenal medullary secretion (41, 46). NE is released into the neuroeffector junctions. Reuptake of NE into the neurons or metabolism by O-methylation after uptake account for a major portion of the released NE and only a fraction of NE released from the sympathetic nerves

reaches the blood (41, 45, 46). 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid; VMA) is the major urinary excretion product of E and NE in humans (41, 45). Metanephrine and normetanephrine are important intermediates in the formation of VMA (41, 45, 46).

Under normal conditions, the 24 hour urinary output of E is 3 to 25 micrograms and of NE is 25 to 45 micrograms (44, 50). However, this output varies during periods of rest and increases with activity (51). A patient at rest excretes 3 to 5 times less NE and 3 to 6 times less E than the patient engaged in moderate activity (49). Under stressful conditions such as severe muscular exercise, centrifugation, trauma, operation, thermal injury, radiation, emotional stress, etc., there is a marked increase in the urinary output of these hormones reflecting an increase in sympathetic nerve and adrenal medullary activity (50, 51).

Urine is the easiest source to analyze for catecholamines. Catecholamine levels in the urine need adjustment for the urinary concentration which can be done by either including total volume of urine, collecting all urine over a fixed time period or by measuring creatinine levels as an estimate of concentration. Creatinine determinations can be done on 24-hour urine samples or on the individual sample and the result equated in micrograms of amine per milligram of creatinine or total volume per unit of time.

Literature Review on Catecholamine and Stress

The relationship of stress and catecholamine excretion is consistent in both non-pregnant as well as pregnant populations. To be able to interpret these

results meaningfully, however, it is necessary to consider the possible contribution of other variables such as collection factors and maternal characteristics variables. There appears to be little consistent information on the relationship between these factors and catecholamine excretions in non-pregnant population and even less information in pregnant population.

Non-Pregnant Population

Levels of Catecholamines in Non-Pregnant Population:

Hansen et al (52, 53) mention about high within and between subject variations in urinary catecholamine excretions for healthy men and women population from Denmark. Within subject variation was found to be more than twice the between-subject variation for E, while for NE the within-subject variation was comparable to the between-subject variation.

Urinary E and NE were positively correlated with range of r=0.14 to 0.76 in non-pregnant women (53-55), national guardsmen and psychiatric patients as subjects (56), bereaved elderly persons (57), male bus drivers (58) and male employees (59). In healthy men and women, correlation between urinary E and NE was 0.41, between E and DA was 0.37 and between NE and DA was 0.37 (60). Mean urinary DA excretion pattern seemed to follow mean NE excretion but not E excretion (61). There was a reported strong correlation between urinary NE and DA excretion in PTSD group r=0.62, p<0.001 (62) and r=0.77, p=0.001 (63), but not a strong correlation in the control group r=0.11, p=non-significant (ns) (63). Urinary E had weak correlation with the other two catecholamines (62).

While yet another study found urinary DA, E and NE to be intercorrelated with Spearman rank correlations ranging from 0.47 to 0.69 (64).

Factors Associated with Catecholamine Levels in Non-Pregnant Population:

Circadian Rhythm- Studies have reported that urinary catecholamine excretion follows a circadian rhythm, with highest excretion in the afternoon and the lowest in mornings and night (52, 53, 60, 61, 65-70). Only study found reduction in urinary E excretion in the afternoon as compared with the morning (71).

Ethnicity- There was no consistency found in literature for association between ethnicity and catecholamines. Pratt et al (72) showed normotensive black children to have significantly lower levels of nocturnal urinary NE than that of white children, while Ziegler et al (73) found white hypertensives had elevated plasma NE levels, and black hypertensives had normal levels. De Bellis et al found African-Americans excreted a significantly greater concentration of urinary E and showed a trend for significantly greater concentrations of urinary NE over 24 hrs than Caucasian subjects (74). Another study found significant ethnicity differences in DA levels; with AFAM individuals having DA levels higher than whites (64). Three other studies found no effect of ethnicity on urinary E and NE (75, 76) and plasma NE (77).

Age- The relationship between age and catecholamine excretion varied across studies. Yehuda et al found no significant correlation between plasma NE and age (78). Hansen et al, Pratt et al and Evans et al did not detect any association between age and urinary catecholamines (52, 58, 72). But a few others found

weak to strong positive association between age and urinary catecholamines (57, 79-84). While two other researchers found that age was significantly negatively correlated with urinary E and NE in men in their fifties and seventies (85) and in children (86).

Education- No significant correlation was found between education and urinary E and NE (58).

Gender- Gender was found by many studies to be an important factor associated with catecholamine excretion. Females tend to have lower excretion of urinary E and NE as compared to men (52, 72, 74, 79, 81, 86-90). Because of sex differences, researchers caution against generalizing results based on men to women.

Blood Pressure- Hypertensives were found to have increased urinary and plasma catecholamine excretion (73, 81, 91, 92). No significant correlation between plasma NE and blood pressure (78).

Smoking- Smoking was found to increase excretion of urinary E and NE (52, 91, 93, 94). Two studies found urinary E and NE not to be associated with smoking (79, 84).

Alcohol- Drinking was positively associated with urinary E (79).

Body Weight- Schmitt et al (79) and Pratt et al (72) found body weight was unrelated with urinary E and NE and Yehuda et al (78) found no significant correlation between plasma NE and weight. Hansen et al (52) found inverse relationship between urinary E and NE and body mass index (BMI), while Jenner et al (80) showed that urinary NE tend to be positively related to body weight and

van Der Beck et al found positive relationship between urinary NE and BMI. Only urinary NE and DA (not E) was significantly correlated with body weight, $r=0.39$, $p<0.05$ and $r=0.46$, $p<0.01$, respectively (95).

Urine flow- Strong and consistent positive correlation was found between urinary E and NE and urine flow (71, 79), while another study found no correlation between urinary E and NE and urine volume (60).

Creatinine- Urinary E and NE were positively correlated with urinary creatinine (60, 71).

Shift Work- Shift work tends to change the pattern of urinary catecholamine excretion (61, 66). Fujiwara et al (65) found urinary NE was significantly higher in the evening shift workers than in the day shift ($p=0.027$), while opposite relation was found by Yamasaki et al (75) and Boucsein et al (96) with higher urinary E and NE in day shift workers than evening and night shift workers. While Cavatorta et al (97) did not find any difference in levels of serum catecholamines at both morning and afternoon shifts.

Parental Status- Though women with at least one child living at home (parental status) reported significantly higher levels of home strain than did women without children at home, an effect that was independent of marital status, home strain and parental status were unrelated to urinary E and NE levels (98).

Marital Status- Nurses who were not married showed a significant decrease in urinary NE on off days as compared to work days, whereas the work and off day levels were similar in married nurses (99).

Urinary E was found to have weak positive association with age in females while negative association was observed in males (79) and urinary NE and E were found to be lower in healthy women than in men in the age group ≤ 39 years of age (i.e. during the first half of life expectancy), while excretion was similar in men and women in the ≥ 40 years of age group (i.e. second half of life expectancy) (81). Creatinine excretion was seen to be decreasing with increasing age (80). Low SES /high pessimism women demonstrated higher mean 24 hr E levels ($p<0.05$) compared with other 3 groups (high SES/high pessimism, low SES/low pessimism, high SES/low pessimism) and low SES /high pessimism had significantly higher 24 hr urinary E/NE ratios than any other group ($p<0.005$) (100). Another study found SES did not predict urinary catecholamine excretion (74).

Relationship between Catecholamines and Stress in Non-Pregnant Population:

Perceived Stress (PS)- Three studies found that perceived stress had a significant positive association with urinary E and NE (82, 101, 102), while three other studies did not find any such relationship (54, 103, 104). Among the negative studies, one did not use a validated method to measure PS (103), another used short term noise stress for which urine might not be a suitable measure of catecholamine (54) and the third study did find lower levels of urinary E and NE in chronically stressed women but these differences did not achieve statistical significance, probably a result of insufficient power (104).

Anxiety- Significant correlations are reported between state anxiety and urinary E, NE and DA (56, 85, 88, 105-107). Correlations ranged from 0.18 to 0.50.

Depression- Depressed subjects had significantly greater amount of urinary E NE and DA excretion, $p<0.01$ to $p<0.007$ (86, 89, 90, 108-110) , while one study found a significant inverse correlation between urinary NE and depression (57).

Work-stress- Work period urinary E and NE excretion was significantly higher than rest period excretion ($p<0.0001$ to $p<0.05$) (53, 55, 67-69, 76, 83, 92, 96, 98, 99, 106, 111). Only one study by Korunka et al did not find urinary E and NE to be significantly different on work and non-work day and also found only a weak relationship between catecholamine levels and subjective strain measurements (59). Higher work stress was associated with higher urinary NE and E excretion (55, 92, 111, 112), while few other studies failed to support this association (76, 83, 98, 99).

Physical Stress- High physical stress urinary and plasma E and NE were significantly higher than baseline levels (68, 70, 83, 113-116).

Other Types of Stress- Other types of stressors which have been examined in literature are bereavement (57), noise (54, 66, 82, 97, 115, 117, 118), traffic congestion (58, 107), crowdedness(119), premenstrual stress (101), weather conditions (107) and dental procedures (120, 121). Mean 24-hr urinary NE and E output for bereaved subjects were significantly higher than those in previous studies of normals (57). Noise exposed subjects had significantly higher urinary E and NE excretions (54, 66, 82, 97, 115) with correlation between perceived noise and E and NE being 0.30 and 0.24 respectively (54). While two studies did

not support this association, first in children exposed to chronic aircraft noise (117), where children with low noise exposure reported significantly more stressful life events, this difference in life experience might account for the lack of noise effects and second in male subjects, probably due to small sample size (118). Traffic congestion had significant positive correlation with on-the-job urinary E and NE elevations (58, 107). Perceived crowdedness was associated with increased urinary NE and E excretion (119). Urinary E was significantly positively correlated and NE significantly negatively correlated with premenstrual turmoil (101). Urinary E and NE excretions were high when weather conditions were stressful such as driving in the fog (107). Urinary E and NE were found to be significantly higher in children after dental procedure and on the experimental day as compared to the control day of dental treatment (120, 121). Increased urinary E and NE excretions were also found with increased mental stress (70, 115, 122), thermal stress (115), fatigue (123), flying in planes (124) and parachute jumping (125). Plasma E declined significantly in the vulnerable elderly Alzheimer spousal caregivers who received respite, but rose in those who were wait-listed (122). Hansen et al found no relationship between urinary E and NE with scales of cognitive, behavioral and emotional stress (53).

Post-traumatic Stress Disorder (PTSD) subjects were found to have significantly higher excretions of urinary catecholamines, E, NE and DA (62-64, 74, 84, 95, 126, 127) and plasma NE (78). Urinary catecholamines were positively correlated with duration of PTSD trauma and severity of PTSD

symptoms (62, 74). Two authors did not find association between urinary E and NE and PTSD (128, 129).

Perceived control appeared to be an important link in the psycho-physiological stress process. This is seen in three studies where elevated urinary E and NE levels were seen only in those subjects where closing the window could not reduce the perceived disturbance due to traffic noise (82), perceived job control had negative correlation with on-the-job E and NE elevations (58) and parents of children admitted to critical care who appraised their child's situation as one that they could change had lower anxiety and significantly lower catecholamine excretion than parents who appraised their child's situation as the one that must be accepted (88).

Briefly, the literature reviewed on catecholamines and stress in non-pregnant population points towards a consistent and strong relationship between these two factors. Several measures have been used to assess stress such as self-report, responses to stress (anxiety and depression) and stressors such as physical stress, work-stress, noise, thermal stress and mental stress. Though studies examining factors associated with catecholamine levels produced mixed results, few variables such as circadian rhythm, age, gender and smoking are found to be steadily associated with catecholamines.

Table 3: Literature Review on Catecholamines in Non-Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Hansen AS/ 2001	Healthy non-pregnant women, Denmark/ N=11	Urinary E and NE from urine samples	High within and between subject variations. E and NE excretion highest during middle of the day and overall average shows evening levels to be higher than morning. E and NE positively correlated within subjects (0.38 to 0.76). NE was higher during work hours (afternoon) and lower during evening hours, except in June and July when NE was lower during work hrs and higher during off hrs. E was higher during June and July compared to rest of the year, maybe due to increase physical activity in summer days. No relationship between E and NE with scales of cognitive, behavioral and emotional stress.
Hansen AS/ 2001	Healthy men and non-pregnant women, Denmark/ N=120	Urinary E and NE from urine samples	Within-subject variation was more than twice the between-subject variation for E. For NE, the within-subject variation was comparable to the between-subject variation. No gender differences for NE, but gender difference present for E (women excreted lower E during the day as compared to men). Reference interval for E *Women:* Morning=Limit of Detection (LOD)-2.10, Afternoon=0.64-10.8, Evening=LOD-8.66; *Men:* Morning=LOD-2.86, Afternoon=1.20-11.19, Evening=LOD-7.79 Reference interval for NE *Women and men:* Morning=3.6-29.0, Afternoon=11.0-54.1, Evening=7.3-49.2 Compared to morning, both afternoon and evening E and NE were significantly higher (afternoon had highest values). No age differences could be demonstrated. Smoking increased excretion of E and NE. Inverse relationship between E and BMI, also for NE and BMI.

Table 3: Literature Review on Catecholamines in Non-Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Schmitt LH/ 1995	Australian aboriginal communities/ N=139	Urinary E from 2 hour (hr) urine sample	Females had significantly lower E than males (P<0.01). E in females had a positive weak association with age, while negative association was observed in males. E positively associated with urine flow rate and drinking (p<0.05), but not associated with weight and smoking.
Fujiwara S/ 1992	Healthy males/ N=6	Urinary E and NE from 4 hrly urine samples collected during day, evening and night shifts	Free NE excretion showed a significant circadian rhythm in all three shifts. The 2-day mean value for 4-hr free NE was significantly higher in the evening shift than in the day shift (p=0.027). The profile for each shift was quite similar to the corresponding 4-h walking, except that the free NE during sleep still remained at a substantial level, whereas obviously the walking dropped to a negligible level. A significant circadian rhythm was also detected in free E during the day and evening shifts. However, free E did not synchronize well with the rest-activity level in the night shift.
Hollister LE/ 1970	Healthy volunteers/ N=10	Urinary E and NE from 2 urine samples collected – 8 am to 12 noon and 12 noon to 4 pm	Strong and consistent positive correlation between E and urine flow. NE positively correlated with creatinine clearance. Reduction in E excretion in the afternoon as compared with the morning.
Pratt JH/ 1992	Normotensive children aged 9-14 yrs/ N=99 (Blacks=50 and whites=49)	Urinary NE from overnight sample	The mean nocturnal NE excretion was significantly lower in blacks than whites (3.10+-0.056 vs. 3.27 +-0.06 ng/mg of creatinine in log scale) at p=0.005. NE excretion was unrelated to age and body weight. Females had slightly lower NE levels than males (p=0.056).

Table 3: Literature Review on Catecholamines in Non-Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Zuspan FP/1979	Non-pregnant women/ N=12	Urinary E and NE from 24 hrs urine	When smoking subjects stopped smoking there was significant decrease in NE (p=0.02) and borderline significant decrease in E (p=0.08). No synergistic effect of oral contraceptives and smoking seen. Both non-smoking/no contraception and smoking/ contraception groups had lower E and NE values. While smoker not taking contraceptives showed two times increase in NE excretion as compared to non-smoker and 50% increase in E excretion.
Faucheux B/ 1976	Healthy men and women/ N=13	Urinary E and NE from urine collected every 4 hrs for 24 hrs	Circadian variation seen in the urinary excretion of DA, E, NE and creatinine, with maximum excretion of all these substances occurring in the afternoon period between 14.30h and 18.00h and minimum excretion in the morning between 4.00h and 5.00h. None of the catecholamines correlated with the urinary volume but NE positively correlated with urinary creatinine (r = 0.28), DA (r = 0.37) and E (r = 0.41). E was positively correlated with DA (r = 0.37) and creatinine.
Jenner DA/ 1987	Volunteer subjects from populations in UK, USA, Nigeria and the South Pacific	Urinary E and NE from 24 hr urine	NE excretion tended to be positively related to age and to body weight. E excretion tended to show little or no relationship with either age or body weight. Creatinine excretion decreases with increasing age.

Table 3: Literature Review on Catecholamines in Non-Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Yamasaki F/ 1998	Non-pregnant female nurses/ N=88	Urinary E and NE from 24 hr urine	Urinary NE and E were higher during work than non-work periods in both racial groups (African-American women and other racial groups) of day shift workers, but in evening + night shift workers the difference was small and in the opposite direction. In regression analyses predicting NE and E from race and work shift, only the effect of work shift was statistically significant.
Grewen K/ 2000	Post-menopausal women/ N=32 Subjects classified into 4 groups by stable pessimistic attributions (high vs. low pessimism) and by SES (high vs. low)	Urinary E, NE and DA from 24 hr urine	Significant SES*pessimism interactions, with low SES /high pessimism demonstrating higher mean for 24 hr E levels compared with other 3 groups ($p<0.05$) and low SES /high pessimism having significantly higher 24 hr urinary E/NE ratios than any other group ($p<0.005$). No group differences in 24 hr NE levels were found. The 4 groups did not differ significantly in reported depression, anxiety and perceived stress. Strong trend for low SES/High pessimism women to have greater prevalence of hypertension (44%) when compared with other three groups combined (18%).
Lehmann M/ 1986	Healthy men and non-pregnant women/ N=265	Urinary E and NE from 24 hr collection	NE (not E) was found to be correlated positively with age in healthy men and women (except pregnant women). NE and E were lower in healthy women than in men during the first half of life expectancy, while excretion was similar in men and women in the second half of life expectancy. In hypertensive individual, catecholamine excretion was slightly higher in the first half and significantly higher in the second half of life expectancy.

Table 3: Literature Review on Catecholamines in Non-Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Ague C/ 1974	Habitual male smokers/ N=24 Each smoked one nicotine –free and three tobacco cigarettes.	Urinary E and NE from 4 hr urine samples from each subject (pre-smoking and post smoking).	No changes were observed in NE excretion. E excretion rose significantly after smoking, although no major differences were found between the treatments.
Aziz MT/ 1977	Male adults/ N=54 (Non-smokers =25 and smokers=29)	Urinary catecholamines from 24 hr urine	The resting level of urinary catecholamines is somewhat raised in the smoker group particularly in the hyperreactors, while is much higher in hypertensive smokers. Smoking 20 cigarettes caused a higher rise in urinary catecholamines in the hyperreactors of both groups than in the normoreactors. Hypertensive smokers exhibited the most exaggerated rise in urinary catecholamines in response to smoking.
Stoney CM/ 1987	Meta-analysis from 7 studies	Urinary E and NE	Relative to females, males had larger urinary E responses during stress ($p<0.003$), but not at rest. No significant sex differences were found for NE.
Rowlands DB/ 1982	Mild to moderate hypertensives/ N=16	Plasma NE	The median resting plasma NE levels of the blacks was 2.02 nmoles/liter (range 0.3 to 6.48 nanomoles/liter) was not different from that of the whites at 1.52 nmoles/litre (range 0.77 to 3.6 nmoles/litre).
Ziegler MG/ 1991	Healthy male/ N=37 (hypertensive=19 and normotensive=18)	Plasma NE and NE clearance	Both normotensive and hypertensive blacks had increased ^{3}H-NE clearance rates ($p<0.001$). Increased NE clearance by blacks may help explain observations that white hypertensives in the age range studied (25-46 years) had elevated plasma NE levels, while blacks have normal levels.

Table 4: Literature Review on Relationship of Perceived Stress and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Babisch W/ 2001	Premenopausal women/ N=195	Overnight urinary E and NE	Higher NE concentration was associated with increasing age. "Objective" noise exposure (traffic volume) was associated with significantly increased NE excretion in noise exposed subjects. No significant association between traffic volume and E excretion. Subjective perception of disturbance due to traffic noise had significant positive association with NE and E excretion. However, this was only found in subjects where closing the window could not reduce the perceived disturbance.
Woods N/ 1998	Non-pregnant women/ N=49	3 hour afternoon urine sample E and NE	Correlations calculated with data from the low symptom severity (LS) and the premenstrual syndrome (PMS) groups indicated that women with higher pre-menses perceived stress had higher NE levels. For women with the LS and premenstrual magnification (PMM) patterns, perceived stress was not related to E and NE excretion. E was significantly positively correlated and NE significantly negatively correlated with premenstrual turmoil when data was analyzed from LS and PMM groups, but this was not the case for analyses with the LS and PMS groups.
Ritvanen T/ 2003	Subjects/ N=51 (full time teachers=17 and control group =34)	24 hour urinary E and NE in four months over a year	The full time teachers reported more perceived stress than the control group. Perceived stress and E levels decreased significantly during summer holidays when the stress was reported to be the lowest as compared to the working days in full time teachers.

Table 5: Literature Review on Relationship of Perceived Stress and Catecholamines in Non-Pregnant Population – Negative studies

Author/ Year	Population/ Sample size	Measurement	Results
Kang J/ 2003	Young males/ N=284	Spot urine E, NE and DA	Urinary concentration of catecholamines did not show any significant correlation with perceived subjective stress in males using PCs in PC game rooms.
Evans GW 2000	Non-pregnant women/ N=40	Urinary E and NE from 3 hrs urine samples collected after resting baseline and experimental sessions	Simulated open-office noise elevated workers' urinary E levels significantly, but elevations in NE levels were not statistically different than working under quiet conditions. E and NE correlation = 0.50 ($p < 0.01$). Perceived noise and E correlation = 0.30 ($p < 0.05$). Perceived noise and NE correlation = 0.24 (not significant; ns). Perceived stress and E correlation = -0.08 (ns). Perceived stress and NE correlation = 0.02 (ns).
Powell LH/ 2002	Pre-menopausal, middle-aged women/ N=40 (chronically stressed =20 and non-stressed =20)	Complete 24 hour, overnight and an evening urine sample	Overnight urine showed chronically stressed women to have lower values of E and NE compared to non-stressed but these differences were not statistically significant.

Table 6: Literature Review on Relationship of Anxiety and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
LaMontagne L/ 1994	Parents of children admitted to critical care/ N=22	Urinary E, NE and DA in 3 hour afternoon urine samples.	All three catecholamines mean excretion levels were significantly higher compared to norms for adults (E=38.9, NE=98.2, DA=506.5 micrograms per total volume). State anxiety was also high in this sample (mean=61.4). All 3 catecholamines correlated positively with state anxiety, E (r=0.18, P=0.4), NE (r=0.32, p=0.2) and DA (r=0.41, p=0.06). Fathers had significantly higher E and DA levels than the mothers. Parents who appraised their child's situation as one that they could change had lower anxiety and significantly lower catecholamine excretion than parents with "accept" appraisals.
Chosy J/ 1970	Study 1: National guardsmen/ N=70 (phobia group=44 and no-phobia group=26) Study 2: Outpatients/ N= 46 (phobia group=31 and no-phobia group=15)	Urinary E and NE from urine samples Study 1: after combat patrol exercise and first morning void Study 2: before and after psychological tests	Study 1: Correlations between urinary E and NE for patrol sample was r=0.31 and sleep sample was r=0.24. Phobia group had significantly higher Taylor manifest anxiety scores and E excretion (not NE) than no-phobia group. Study 2: Similar results as study 1. Correlation was r=0.50.

Table 6: Literature Review on Relationship of Anxiety and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Sanders K/ 1999	Non-pregnant women/ N=34	12 hour overnight urinary E and NE	Higher excretion of NE was associated with higher state anxiety ($r=0.43$, $p<0.001$) and less favorable moods on all Bi-polar Profile of Mood States scales (r between -0.22 and -0.39, $p<0.05$). E excretion was not associated with any psychological test scores.
Faucheux B/ 1983	Male subjects/ N= 48 (men in fifties=24 and men in sevenities= 24)	Urinary E and NE before, during and after mental stress	Age was significantly negatively correlated with urinary E and NE. Fifties group responded to stress by significant increase in E and no increase in NE, while seventies group showed a significant increase in both E and NE during mental stress. Several anxiety indices were positively correlated with E and not with NE excretion.
Raggatt P/ 1997	Long distance bus drivers/ N=10	Urinary E and NE from 3 hr urine sample before and 4 hr sample after shift	Excretion rates on driving days were significantly higher for E ($p<0.01$) and NE ($p<0.05$) compared to rest days. Self-reported stress and state anxiety were elevated only in the pre-shift measure.
Vivoli G/ 1993	Long distance truck drivers/ N=3	Urinary E and NE from 4 hourly urine samples	Significant positive correlation was found between urinary E levels and state-anxiety scores ($r=0.50$, $p<0.02$). E excretion was high when weather and traffic conditions were more stressful. NE increased at the end of work day and while driving in fog.

Table 7: Literature Review on Relationship of Depression and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Van Doornen LJP/ 1987	Students/ N=52 (females=23 and males =29)	Urinary E and NE from samples after 10 minutes session on control and examination day.	State anxiety increased significantly in both sexes (males more anxious than females) from control to examination day. State depression was significantly higher on examination day, but no sex differences were seen. On examination day, in males E excretion increased while in females it decreased leading to males having significantly higher E than females. Examination did not influence NE levels in either sex. NE levels higher in females than males nearly reached significance on both days.
Jacobs S/ 1986	Middle-aged and elderly persons/ N=59 (acutely bereaved=39 and threatened with loss of a spouse=20)	Three successive 24 hr urinary samples collected for E and NE	Mean 24-hr NE and E output for bereaved subjects were significantly higher than those in previous studies of normals. The groups did not differ significantly in the catecholamine output. In bereaved group: E and NE significantly correlated ($r=0.65$, $p<0.00001$). Both E and NE significantly correlated with age ($r=0.35$, $p<0.05$ and $r=0.46$, $p<0.01$ respectively). Inverse correlation between NE and depression score remained significant even after adjusting for age ($r= -0.37$, $p<0.05$), while the significance for E ($r= -0.36$, $p<0.05$) was lost after adjusting for age ($r=-0.30$, ns).
Roy A/ 1986	Subjects/ N=53 (depressed patients=28 and normal controls=25)	Two 24 hr urine samples collected for NE and DA	Depressed group had significantly greater amount of NE excretion ($p<0.007$) than controls. Females excreted lower amounts of NE ($p<0.06$) than males. DA did not differ significantly between depressed and control group.

Table 7: Literature Review on Relationship of Depression and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Queiroz E/ 1991	Children aged 7 to 14 yrs/ N=43	First morning urine NE	NE was significantly higher in depressed males compared to non-depressed males ($p=0.05$). Non-significant decrease in NE is seen in females. Age in both sexes negatively correlated with NE.
Grossman F/ 1999	Subjects/ N=34 (bipolar depressed =12, unipolar depressed =10 and healthy volunteers=12)	24 hr urinary NE	Average 24-hr urinary excretion of NE was significantly higher in the bipolar depressed patients than in healthy volunteers ($p<0.05$). Though unipolar patients also had higher NE than volunteers this difference was not statistically significant.
Swann A/ 1999	Depressed subjects/ N=132 and control group N=?	Four consecutive day 24-hr urine NE	Depressed mood and anxiety was significantly higher in both unipolar and bipolar depressed patients when compared to controlled subjects. Urinary excretion of NE was significantly higher in the unipolar depressed patients than in control group. Though bipolar patients also had higher NE than controls this difference was not statistically significant.
Komori T/ 1995	Depressive patients treated with citrus fragrance (CF) N=12, depressive patients treated with anti-depressants (AD) N=8 and non-depressed control group N=20	24 hr urinary E, NE and DA	NE and E did not significantly differ between the groups. Mean DA levels in urine were significantly higher in the CF and AD groups compared to controls ($p<0.01$) before treatment. Mean DA levels decreased after treatment, but DA significantly lower in CF group as compared to AD group.

Table 8: Literature Review on Relationship of Work Stress and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Elfering A/ 2002	Nurses/ N=24	Urinary E and NE in 3 urine samples on workday and day-off	Higher E and NE release was found in the nurses who experienced less control over stressful events at work and reported more exhaustion and worse mood after work.
Brown DE/ 2003	Women nurses and nurse's aides/ N=59 (Filipino-Americans=36 and Euro-Americans=23)	Urinary NE and E from two 4 hr urine samples- work and home and one 8 hr overnight sample	E and NE excretion rates were significantly higher at work than in other daily settings. No significant ethnic differences in catecholamines were found in any settings. E and NE were not significantly different between the group with job strain and not having job strain for any of the periods. Catecholamine excretion rates in the workplace were significantly related to blood pressure variability throughout the day and to SBP means.
Boucsein W/ 1996	Male students/ N=24	Urinary samples E and NE	Both E and NE excretion rates were slightly higher under day-shift as compared to night-shift and lower during sleep as compared to work. An interaction between shift and noise was only present on the 2nd day, where noise raised the E level in night-shift but not in day-shift workers.
Van der beck A/ 1995	Male lorry drivers/ N=32	Urinary E and NE from 6 urine samples on work and rest days	For all samples, except the overnight sample, the excretion rates of both catecholamines on the working day were higher than those on the rest day. Age was positively related to the E excretion rate on the working day ($p<0.05$). BMI and physical workload were positively related to NE (both $p<0.01$). Psychosocial job strain was not related with catecholamine levels.

Table 8: Literature Review on Relationship of Work Stress and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Dutton L/ 1978	Subjects/ N= 36 (firemen=16 and paramedics=20)	24 hr urinary E and NE on work and non-work day	Significantly higher level of job stress was observed in paramedics as compared to the firefighters. On working day, the mean levels of E and NE were approximately 5% higher and on non-working day NE was 17% lower and E was 2% higher for paramedics than firefighters. For paramedics, work-day urinary E and NE levels were elevated by 37% ($p=0.04$) and 15% respectively, as compared to non-workday. For firefighters, work-day urinary E was elevated by 35% and NE levels decreased by 4% as compared to non-workday (urine collection corresponded to a particularly light work load for firefighters).
Luecken L/ 1997	Working women/ N=109	Urinary E and NE from three 8 hrs samples during a workday	Significant increase in catecholamine levels during the workday period and no change from workday period to evening levels. Women with children living at home reported significantly higher levels of home strain than did women without children at home, and effect that was independent of marital status. Work and home strain and parental status (at least one child living at home) were unrelated to E and NE levels.
James GD/ 1993	Non-pregnant women/ N=80 (work-stressed women=45 and home-stressed women=35)	Urinary NE and E from 4 hour samples at work and home and an overnight urine collection	Patterns of catecholamines were same in both groups (highest catecholamines at work gradually decreasing at home and then in sleep E and NE had lowest values), although the absolute values of NE were higher (but not statistically significant) in the work-stressed women. E and NE were positively associated with blood pressure in work-stressed women and there was a greater proportion of women with history of hypertension in the work-stressed group ($0.05<p<0.1$).

Table 8: Literature Review on Relationship of Work Stress and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Sluiter JK/ 1998	Long distance coach drivers/ N=10	Urinary E and NE from 3 hrly urine samples on 3 workdays and on 2 non-workdays	Circadian rhythm for E and NE with midday (early afternoon) peaks, except 2nd workday when rhythm was disturbed because of the daytime sleeping (on 2nd workday peaks were earlier than other workdays around 11 am). The mean excretion rates of E on the first working day and most samples on all working days were higher than the baseline. The overall mean excretion rate of NE on the 1st working day was marginally significantly higher than the overall excretion rate on both days off. For both E and NE the mean excretion rates on the first day off were lower than the baseline.
Lundberg U/ 1999	Female supermarket cashiers/ N=72	2 hr urinary sample E and NE after work period, relaxation period and corresponding times on work-off day	Correlation between NE and E=0.34, while between self-reported stress and NE, E correlations were 0.17 and 0.23 respectively. Stress and tenseness increased significantly during work period compared with the rest period. E and NE (both $p<0.0001$) were significantly elevated during the 2 hr work period compared to the relaxation period at work. Catecholamine levels after work did not differ significantly from the levels measured on the work free day at home.
Goldstein I/ 1999	Registered nurses/ N=138	2 hr urinary E and NE on 2 work days and 2 off days	Values were higher during the work-day than off day for E levels ($p<0.0001$). No difference between high and low job strain for excretion of E and NE. Nurses who were not married showed a significant decrease in NE on off days as compared to work days, whereas the work and off day levels were similar in married nurses.

Table 8: Literature Review on Relationship of Work Stress and Catecholamines in Non-Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Jenner DA/ 1980	Males/ N=413	Urinary E and NE from 3 samples – first morning void, mid-day and evening	Circadian rhythm is seen in catecholamine excretion with highest levels in the afternoon and evening levels being higher than morning levels. E is significantly higher on workday compared to a non-working day in afternoon and evening, while NE is significantly higher only in evening samples. Workday afternoon and evening E levels are significantly higher in non-manual workers compared to manual workers. While manual workers have significantly higher NE output than non-manual workers over the workday afternoon period.
Sluiter/ 2000	Male garbage collectors/ N=115	Urinary NE and E from 5 samples during workday and non-work day	E showed circadian rhythm on both days ($P<0.05$), but NE had circadian rhythm only on workday ($p<0.01$). The overall mean excretion rates of E and NE on the workday were significantly higher than on the rest day ($P<0.01$).

Table 9: Literature Review on Relationship of Work Stress and Catecholamines in Non-Pregnant Population – Negative studies

Author/ Year	Population/ Sample size	Measurement	Results
Korunka C/ 1996	Male employees/ N=14	Urinary E and NE from 6 urine samples during 2 consecutive work-days and one rest day for all 3 phases of the study	A significant correlation was observed between E and NE in phase 1 ($r=0.72$, $p<0.01$) and phase 3 ($r=0.70$, $p<0.01$) but not in phase 2 ($r=0.14$). E and NE not significantly different on work and non-work day. Weak relationship between catecholamine levels and subjective strain measurements.

Table 10: Literature Review on Post-Traumatic Stress Disorder and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Glover DA/2002	Non-pregnant women/ N=29 (mothers of pediatric cancer surviors with PTSD=14 and without PTSD symptoms=7, control mothers of healthy children=8)	Overnight 12 hr urinary E and NE	Trend was seen with elevated NE (microgm/12 hr) for PTSD subjects when compared to all non-PTSD subjects (p=0.07). There was no significant group effect when all 3 groups were kept separate (p=0.18). This trend of NE was duplicated when NE microgram/gm of creatinine was analyzed (3 group, p= 0.15 and 2 group, p=0.06), while with NE as microgm/L both 3 group (p=0.06) and 2 group (p=0.02) effect were significant. No significant 2 or 3 group differences in urinary E. When controlled for depression significant group differences for NE disappeared. NE microgm/L levels were significantly higher for depressed PTSD mothers than for non-depressed PTSD mothers (p=0.05).
Lemieux AM/ 1995	Women/ N=28 (PTSD related to childhood sexual abuse=11, sexual abuse without PTSD=8, non-abused controls=9)	Urinary E, NE and DA from 24 hour urine	PTSD+ group had elevated daily levels of NE ($p<0.05$), E ($p<0.01$), DA ($p<0.08$) and cortisol ($p<0.05$). When depression variables were controlled these group difference still remained. An increased incidence of polyuria was seen in abused population when compared to non-abused. Only NE and DA (not E) significantly correlated with body weight ($r=0.39$, $p<0.05$ and $r=0.46$, $p<0.01$, respectively).

Table 10: Literature Review on Post-Traumatic Stress Disorder and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Hawk LW/ 2000	Subjects/ N=77 (involved in serious motor vehicle accidents, MVA=55 and age matched controls =22)	Urinary E and NE from all urine excreted between 6 pm to 9 am	Preliminary analysis showed older participants tended to have higher E and NE excretion rates at month 6 ($r = + 0.24$ and $+ 0.21$, $p<0.04$ and 0.07 for NE and E, respectively). Greater number of foods on the food checklist was associated with higher rates of E excretion at month 6 ($r = + 0.23$ $p<0.03$). Cigarette smoking, caffeine consumption and injury severity scores were not reliably related to hormone levels (all $\lvert r \rvert < 0.18$, all $p > 0.11$). Catecholamines were related to PTSD diagnosis and symptoms, but only among men. PTSD-symptomatic men who had been in an accident exhibited higher levels of E and NE 1 month after the accident and had higher E levels 5 months later. These effects were not significant among women. At month 1, men in MVA/PTSD group had significantly higher values of E ($p=0.08$) and NE ($p<0.01$) when compared to MVA/PTSD-free group. Lower E ($p<0.01$) in MVA/PTSD-free women when compared to controls and E was lower in MVA/PTSD women when compared to controls. No difference for NE in women. At Month 6, men in MVA/PTSD group had significantly higher values of E ($p=0.06$) when compared to MVA/PTSD-free and for NE this effect was no longer evident. While in women, both E and NE were lower in MVA group when compared to controls (no difference between MVA/PTSD and MVA/PTSD free groups for E or NE).

Table 10: Literature Review on Post-Traumatic Stress Disorder and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Kosten TR/ 1987	Male inpatients/ N=44 (PTSD=9, major depressive disorder(MDD)=8, bipolar manic(BP)=8, paranoid schizophrenia(PS)=12, undifferentiated schizophrenia(US)=7)	Urinary E and NE (24-hr urine at hospital admission and at 2 week intervals thereafter)	The mean NE levels during hospitalization was significantly higher in PTSD (76 ± 0.4 µg/day) than in BP (60.6± 8.4 µg /day), MDD(41.2 ± 4.7 µg /day), PS(33.4 ± 4.9 µg /day) and US(34.3 ± 5.9 µg /day) groups ($p<0.0003$). The mean E level during hospitalization was also significantly higher in PTSD (22.7 ± 2.4 µg /day) than in MDD (13.6 ± 1.7 µg /day), PS (14.7 ± 2.4 µg /day), and US (18.9 ± 1.8 µg /day), but not higher than in BP (21.5 ± 2.7 µg /day).
De Bellis MD/ 1999	Pre-pubertal children/ N=52 (PTSD=18, non-traumatized children with over-anxious disorder (OAD)=10 and healthy controls =24)	Urinary E, NE and DA from 24 hour urine	PTSD group associated with greater depressive and dissociative symptoms. Significantly increased excretion of all 3 catecholamines in PTSD group compared to OAD and control subjects. Catecholamines positively correlated with duration of PTSD trauma and severity of PTSD symptoms. NE and E were found to be higher in males than females. SES did not predict catecholamine excretion. African American excreted significantly greater concentration of E and showed a trend for significantly greater concentrations of NE over 24 hrs than Caucasian subjects.

Table 10: Literature Review on Post-Traumatic Stress Disorder and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Spivak B/ 1999	Subjects/ N=27 (male combat-related PTSD patients (CR-PTSD)=17 and normal control subjects =10)	24 hour urinary excretion of NE and DA	Strong association in the Cr-PTSD group between 24 hr urinary NE and DA excretion (r=0.77, p=0.001), but not in the control group (r=0.11, p=ns). Depression, anxiety scores and 24 hr urinary excretion of NE and DA were significantly higher in CR-PTSD patients than the control group.
Yehuda R/ 1992	Male subjects/ N=38 (male Vietnam combat veterans with PTSD =22 and non-psychiatric normal men =16)	Urinary E, NE and DA from 24-hr urine	PTSD inpatients had significantly higher excretion of all three catecholamines compared with both outpatients with PTSD and normal controls. DA and NE, but not E, levels were significantly correlated with severity of PTSD symptoms in the PTSD group as a whole. None of the catecholamines were correlated with severity of depression. Strong trend for correlation between DA and NE excretion in PTSD group (r=0.62, P<0.001). E had weak correlation with the other two catecholamines.
Yehuda R/ 1998	Subjects/ N=40 (men with PTSD=15, major depressive disorder (MDD) =12 and non-psychiatric comparison subjects =13)	Plasma NE every 30 minutes for 24 hr period	NE levels were significantly associated with severity of depression in the PTSD group (r=-0.85, p=0.002), but not the MDD group (r=0.21). When the three groups (PTSD with depression, PTSD without depression and MDD) were compared to the healthy comparison group, only the PTSD group without secondary depression showed significantly higher plasma NE levels (p<0.01). No significant partial correlations (controlling for group) were found between NE and age, weight, height, blood pressure and pulse.

Table 11: Literature Review on Post-Traumatic Stress Disorder and Catecholamines in Non-Pregnant Population– Negative studies

Author/ Year	Population/ Sample size	Measurement	Results
Pitman/ 1990	Male combat Vietnam veterans/ N=23 (PTSD = 13 and healthy = 10)	24 hour urinary NE and E before and after a stressful combat related experiences interview	No significant group differences were found (p=1.0).
Mellman/ 1995	Vietnam veterans/ N=30 (PTSD combat veterans= 20 and controls non-combat veterans = 10)	24 hour urinary NE and E	No significant differences in 24-hour or "nocturnal" NE or "nocturnal minus daytime" NE between PTSD patients and controls.

Table 12: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Evans GW/ 1991	Male bus drivers/ N=60	8 hrs overnight and on-the-job urinary E and NE	Overnight E and NE had poor correlation of 0.18 with each other but on-the-job E and NE correlation was significant at 0.43. No correlation between age, education and neither E nor NE. Traffic congestion had significant positive correlation, while perceived control had negative correlation with on-the-job E and NE elevations. Perceived job control appeared to be an important link in the traffic congestion-psycho-physiological stress process.
Lundberg U/ 1976	Subjects/ N=17 (group 1- boarded train when less crowded=8 and group 2 boarded train when crowded=9)	Urinary E and NE in samples collected after train trip and after 2 hrs of relaxation at home	No significant difference between groups for catecholamines at home. Perceived crowdedness increased as the square of the number of passengers per car. NE and E excretion was significantly higher in group 2 who boarded the train when it was crowded than group 1. E excretion was higher in both groups during trip 2 (when the train was more crowded) than trip 1 (when the train was less crowded), while there was no difference in NE excretion between the 2 trips.
Vaananen I/ 1997	Finnish soldier participants of 4 day march/ N=6	Urinary E and NE from samples of urine collected overnight before and between marching days and 4 samples collected during each day march	Subjects underwent a period of extraordinary heavy physical stress period during the march which was accompanied by cumulative sympathoadrenal stress as seen by the cumulatively increased E and NE excretion during the successive marches.

Table 12: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Hijzen T/ 1984	Male students/ N=27	Three 1 hrly urine and 2 blood samples before and after workloads on bicycle ergometer	During high physical stress urinary and plasma NE and plasma E are significantly higher than baseline levels and levels during low physical stress. No significant difference found for urinary DA levels.
Sakuma N/ 1996	Children of age 3 to 5 yrs/ N=7	Urinary E, NE and DA before and after dental procedure	NE and E concentrations after dental treatment were significantly higher than before the treatment (mean increases of 51% and 231% respectively). No changes seen for DA concentration.
Alessoandro C/ 1992	Children ages 5 to 8 yrs/ N=20	Urinary E and NE from 24 hrs urine before wait and 1 hr after wait in waiting room in dental office on control (very first dental visit in life) and experimental day (second dental visit)	Experimental day visit had significantly higher urinary E and NE excretion than control day.

Table 12: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample Size	Measurement	Results
Cesana G/ 1982	Subjects/ N=45 (group 1 exposed to noise and shift workers=15 pressmen, group 2 shift workers and not exposed to noise=15 maintenance men and group 3 not exposed to noise and not shift workers=15 clerical staff)	Three 8 hrs urine sample E and NE	NE excretion in group 1 is greater than that of group 2, which in turn is greater than group 3. E excretion is similar in group 1 and 3 and greater than group 2. In group 3, E excretion shows a circadian pattern with lower levels at night and higher in the afternoon, but for NE circadian pattern is less evident because levels at night are similar to in the afternoon and higher than morning values.
Cesana G/ 1982	Subjects/ N=45 (group 1 exposed to noise and shift workers=15 pressmen, group 2 shift workers and not exposed to noise=15 maintenance men and group 3 not exposed to noise and not shift workers=15 clerical staff)	Three 8 hrs urine samples DA	DA excretion is low in morning, reaches a peak in afternoon, maintaining high values during the night. Shift work seems to flatten this pattern with increasing the mean morning excretion levels. Mean DA excretion pattern seems to follow mean NE excretion but not E excretion.

Table 12: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample Size	Measurement	Results
Tsaneva N/ 1975	*Group 1:* Professional typists/ N=8 (typing at slow speed=4 and typing at high speed =4) *Group 2:* Interpreters doing foreign translations exposed to 3 intensities of noise stimulus/ N=5 *Group 3:* Students before, after and during examination sessions/ N=8 *Group 4:* Miners working at temperature of 26 and 28 degree Celsius/ N=29	Urinary E and NE from urine samples	*Group 1:* Slow and normal speed of typing did not change values of E but a significant increase in NE was observed. *Group 2:* Increase in the values of E was proportional to the intensity of the sound (p=0.02), but NE increased only when intensity of sound reaches 85dB. *Group 3:* E showed a twofold increase (p=0.001) after exam while NE which was very high before examination, increased by 30% after the examination (p=0.01). *Group 4:* Compared to many other comparison groups and values regarded as normal for E and NE, miners had significant increase in NE levels.
Froberg J/ 1970	Female invoicing clerks/ N=12	Urinary E and NE	Increased E and NE were observed with increased fatigue.

Table 12: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample Size	Measurement	Results
Deroanne R/ 1975	Parachute jumpers/ N=6	Plasma and urinary NE and E before and after jumping	No significant difference was measured in plasma E or NE levels before and 1 hour after the jump. Urinary E and NE increased significantly after the jump (p=0.02 and 0.005 respectively). Urinary E (230% of reference value) and NE (156% of reference value) after jumping were significantly higher than resting values measured in 13 normal medical students.
Cavatorta A/ 1987	Healthy male workers/ N=112 (group 1 exposed to high environmental noise levels=60 and group 2 exposed to low environmental noise levels=52)	Serum E, NE and DA at beginning and middle of morning and afternoon shift	Baseline values in group 1 and 2 subjects were nearly equivalent. NE and E were significantly increased in group 1 (about 70%) at mid-shift, with respect to baseline and group 2 values. Increases in catecholamines were nearly equivalent in group 1 at both morning and afternoon shifts. Serum DA showed no significant differences.
Von Euler US/ 1954	Subjects/ N=27 (group S: military privates with no flying experience were transported in planes as passengers=14. and group B: Pilots with flying experience practicing routine flights=13	Urinary E and NE from urine samples from 7 to 11 am; 10 pm to 7 am from group S	In group S, the E output during ground activity (6.7 ± 0.98 mmicrogm/min) and during flight (24 ± 2.7) was significantly different (p>0.001), while NE output was not significantly different. Within group S, the subgroup with flight the following day had significantly higher E (no significantly higher NE excretion) in the overnight urine sample compared to the subgroup without flight the following day. In group B, both E and NE excretions were significantly higher during flying as pilots as compared to when performing ground activity.

Table 12: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample Size	Measurement	Results
Sudoh A/ 1971	*Experiment (Exp)1:* Subjects/ N=3	24 hr urinary NE and E	*Exp 1:* The catecholamine values were high in daytime and low at night.
	Exp 3-i: Males/ N=16	Urine samples NE and E - control, test and recovery urine	*Exp 3-i:* Significant increase in E after continuous arithmetic calculation ($p<0.05$), but slight increase of NE excretion observed during the test is not significant.
	Exp 3-ii: Males/ N=2		*Exp 3-ii:* Repeating the same task for 5 consecutive days did not show any adaptation to calculation stress, as seen from increase in E excretion of all 5 days.
	Exp 3-iii: Males/ N=11		*Exp 3-iii:* 2 days of maximum calculation speed and 3rd day of usual speed.About 150% increase in E output was observed on maximum speed calculation days only ($p<0.01$), while the increase in E at usual speed was not significant. In the first day, NE levels were significantly increased ($p<0.05$), but not on the other two days.
	Exp 4: Workers/ N=12 (group A, monotonous light work=4, group B, moderate physical work=4 and group C, heavy physical work=4	Urine samples NE and E – morning, afternoon and night	*Exp 4:* In group A, E was increased by 30% and a decrease in NE was observed. NE increased by 60 % in group C.

Table 12: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Positive studies

Author/ Year	Population/ Sample Size	Measurement	Results
Baker JS/ 2003	Healthy male students/ N=18	Plasma E and NE (10 ml blood collected pre, immediate post and 4 hr post-exercise)	Increase in blood concentration of E and NE ($p<0.05$) were recorded for both total body mass and fat free mass protocol immediately post exercise. These post exercise concentrations return to resting levels 24 h post exercise.
Grant I/ 2003	Elderly Alzheimer spousal caregivers/ N=55	Plasma E and NE	At the one month follow-up, plasma E declined significantly in the vulnerable caregivers who received respite, but rose in those who were wait-listed. No effect was found for NE or psychological symptoms. Experimental speech stressor task raised both E and NE significantly.

Table 13: Literature Review on Relationship of Other Types of Stress and Catecholamines in Non-Pregnant Population– Negative studies

Study (Author/ Year)	Population (Sample size)	Measurement	Results
Haines MM/ 2001	School children/ N=204 (exposed to chronic aircraft noise=96 and controls=108)	Overnight 12 hour urine NE and E	The high and low noise exposed children did not differ in secretion of E and NE and did not differ in perceived stress. But children with low noise exposure reported significantly more stressful life events, this difference in life experience might account for the lack of noise effects on perceived stress.
Miki K/ 1998	Male subjects/ N=8	Urinary E, NE and DA before, during and after arithmetic calculations under quiet and noisy conditions	The scores for "feeling irritable" after the task with noise were significantly higher than that after the task without noise. Marked statistically significant E excretion was observed while performing arithmetic calculations in a noisy environment ($p<0.05$) and under quiet conditions ($p<0.05$) compared with the respective pre-task levels. The E excretion increments under both conditions were almost the same. NE showed a non-significant tendency to increase during the task. No task-induced change was observed in DA excretion.

Pregnant Population

Levels of Catecholamines in Pregnancy:

Studies done on pregnant women have shown urinary excretion of E and NE during pregnancy to remain essentially within the same ranges as normal, non-pregnant subjects (14, 15, 130-137). Urinary NE excretion ranged from 6.2 to 56.4 micrograms (μg) per 24 hrs, while E ranged from 0.18 to 18.1 μg per 24 hrs (131). These ranges were compatible with the levels found by Subrahmanyam (133) in urine of normal pregnant women (NE=36.2±1.18 μg and E=6.5±0.37 μg) and non-pregnant women (NE=33.7±1.62 μg and E=5.7±0.54 μg). Lederman et al found plasma values of catecholamines in third trimester to be E=39.8±20 picogram/ml and NE=212.4±60.1 picogram/ml (137). These excretions of E and NE which remain within normal limits during the course of pregnancy start to rise with the onset of labor and drop down gradually during the postpartum period (14, 15, 130, 132, 137). Most of the studies collected urine over a 24 hour period for determination of E and NE excretion (14, 130-133), while two studies were based on plasma levels of E and NE (15, 137).

One study (138) found NE and E to be positively correlated with each other ($r=0.42$, $p<0.03$).

Factors Associated with Catecholamine Levels in Pregnancy:

Circadian Rhythm- Urinary catecholamines showed a circadian pattern with a significant rise of NE and DA levels in the afternoon period and lower E levels in the night period at $p<0.05$ (139).

Age- The positive correlation seen between urinary E and age $r=0.41$, $p<0.04$ (138) could indicate that the older women were experiencing more stress with greater demands from children and other responsibilities, while another study did not find any significant correlations between maternal age and urinary catecholamine (E, NE and DA) measures (140).

Education- Education and urinary catecholamines (E, NE and DA) did not have any significant correlations (140).

Gestational age- Gestational age was not associated with the rise in plasma NE during physical stressful stimulus of puncturing the fetal trunk for blood sampling or transfusion (141).

Posture- Posture was shown to influence excretion of catecholamines, in urine samples collected after 40 minutes in recumbent position and 40 minutes of ambulation. NE levels significantly increased ($p=0.006$) when the lateral recumbent position was compared with post-ambulation pre-stocking period, there was marginal increase in DA and the increase in mean E was not statistically significant (142).

Blood Pressure- Toxemic patients had increased urinary NE and E excretion when compared with normal pregnant women (133, 134) and normal women (133). The increases in the plasma levels of NE and E after submaximal exercise test showed a positive correlation with the increase in systolic blood pressure, $r=0.75$, $P<0.05$ and $r=0.75$, $P<0.05$ respectively (143). Nisell et al found plasma NE and E levels at rest and after mental stress test were not significantly different

between the group of women with pregnancy induced hypertension and healthy pregnant controls (144).

Altitude- Women residing at high altitude tend to have higher levels of catecholamines during pregnancy than women living at lower altitude (140).

Relationship between Catecholamines and Stress in Pregnancy:

Among the 20 studies reviewed, 17 studies showed a consistent relationship between stress and catecholamine excretion in pregnancy (135-138, 140, 141, 143-152), while 3 studies did not support this relationship (139, 153, 154). Studies have been done on different populations such as low socioeconomic class, African-Americans, adolescent women and physicians.

Anxiety- Field et al (145) found high anxiety pregnant women to have significantly elevated urinary NE ($p=0.05$), elevated E (not statistically significant) and low DA ($p=0.01$) levels. While Kemp et al (138) did not find any significant correlations between urinary NE and E with state anxiety. Study by Sanders et al found a significant inverse relationship between urinary NE and composed-anxious scale, $r = -0.52$ and $p<0.05$ (153). High anxiety women were found to have higher scores on depression and anger scales (145). High-risk pregnancy group among low socioeconomic pregnant women had significantly higher levels of E levels in urine compared with low risk group, though there were no significant group differences for anxiety scores (138).

Depression- While one study found significantly higher excretion of urinary NE, E and DA levels during neonatal period in mothers with depressive symptoms and their infants and significant correlation of Beck depression inventory scores at 6

months post-partum with elevated NE at neonatal period (146), other two studies found significantly lower levels of plasma NE and E on day with post-partum blues (152) and statistically significant correlation ($r=0.76$) between decreased urinary NE and increased depressive scores in pregnant women and non-significant increase in E (149).

Work Stress- Both E and NE excretion in urine were significantly higher (52 to 93%) on workdays than non-workdays (135, 147, 148). State anxiety test taken midway through work and non-work days showed a median 34% increase on workday compared to non-workday (147). Elevated risk of poor pregnancy outcomes such as preterm delivery, low birth weight are seen in practicing physicians and nurses, which may be related to high levels of psychological stress and long working hours in these professions (155). Studies have shown urinary E and NE to be increased by 64% ($p<0.025$) in physician/nurse group during workdays over those of a working non-physician control group of similar gestational age (148). Low job latitude was significantly associated with a slight increase in urinary NE and DA levels in the afternoon and night, $p<0.05$; however no significant increase in catecholamine levels was found in association with the other indices of life stress or work stress, although NE values tend to be higher with most stress measures (139).

Physical Stress- Measurements of urinary excretion of vanilmandelic acid (metabolite of E and NE) before and after several forms of exercise found levels to be higher after exercise, though this difference did not achieve statistical significance (134). Rauramo et al (143) were able to support this theory by

showing plasma concentrations of NE and E rising rapidly during sub-maximal exercise, from 2.9±0.3 to 6.9±1.2 nmol/l and from 0.31±0.04 to 0.47±0.08 nmol/l, respectively and then declined rapidly thereafter.

Other Types of Stress- Four studies examined levels of catecholamines in plasma of pregnant women after stressful events such as hooked to fetal heart rate monitor (151), exposure to thermal stress (136), standardized mental stress test (144) and undergoing fetal blood sampling or transfusion (141). Plasma NE and E rose significantly in pregnant women not in labor hooked on fetal heart rate monitor (Group A), in comparison with the group of pregnant women not hooked on fetal heart rate monitor (Group B), after start of monitoring and began to fall after monitoring was cut off at 30 minutes. In group B, there was no rise in E and NE. No difference was seen in DA levels between the two groups (151). Fetal heart rate monitor might be a source of stress for group A women. Exposure to thermal stress and mental stress test induced feeling of discomfort, stress and irritation. Both stresses caused levels of plasma NE and E to be significantly elevated during and after exposure (136, 144). Women undergoing fetal transfusion in second and third trimester had substantially elevated plasma NE levels after transfusion (141). Fetal plasma NE levels also showed a significant increase after transfusion, with significantly higher levels in those fetuses with samples obtained via intra-hepatic vein (IHV) than in fetuses in whom samples were obtained via placental cord insertion (PCI) site (141). This difference might be due to the fact that IHV is innervated and PCI is not innervated leading to dissimilar physical stresses felt by the fetuses, supporting the other finding of

duration of stimulus (i.e. the time interval from puncturing the fetal trunk to collecting the last sample) being associated with the rise in NE in the IHV group (141).

Two intervention studies (146, 150) strengthen association between catecholamines and stress. Depressed mother subjected to intervention of 3 months had significantly lower mean Beck depression inventory scores at 6 and 12 months after intervention and significantly lower urinary E and DA levels than depressed mothers not provided with intervention (146). Pregnant women with massage and relaxation therapy reported feeling less anxious after therapy (150) with significant decrease in urinary NE ($p<0.01$) for massage group and increase in urinary DA for massage group ($p<0.01$) and for relaxation group ($p<0.005$) as compared to catecholamine levels before therapy.

The 15 minutes experimental noise stimulation did not induce changes of plasma E and NE in pregnant normotensive and hypertensive women (154).

Compared to non-pregnant population the range of studies done on pregnant women examining the relation between catecholamines and stress is limited, still consistent associations are seen. Research on factors associated with catecholamine levels in pregnancy is very restricted and more studies are required in this area to make reliable conclusions.

Table 14: Literature Review on Catecholamines in Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Goodall McC/ 1971	Pregnant women/ N=20	24 hrs urinary E and NE	Urinary output of E and NE remain within normal limits during the course of pregnancy until the onset of labor. Concomitant with onset of labor, marked increase in output of E, NE (especially in NE) and after delivery gradual increase in both with highest levels at 6 to 18 hr postpartum period.
Zuspan FP/ 1970	Women/ N=12 (pregnant women=6 and normal non-pregnant women of childbearing age=6)	24 hrs urinary E and NE	Antepartum, postpartum and non-pregnant values were essentially same for E and NE. Trend towards increased exertion of E 24 hrs after delivery, but was not statistically significant. 24 hr NE exertion 24 hrs after delivery was significantly higher (2 to 3 times) than antepartum or postpartum levels.
Jaffe RB/ 1969	Pregnant women with uncomplicated pregnancies/ N=22	24 hrs urinary E and NE between 20^{th} and 39^{th} weeks of gestation	NE ranged from 6.2 to 56.4 micrograms per 24 hrs. E ranged from 0.18 to 18.1 micrograms per 24 hrs.
Zuspan FP/ 1967	Normal pregnant women/ N=4	24 hr urinary E and NE	No significant difference was found when antepartum values were compared each week to antepartum values or when postpartum values were compared each week to postpartum values.

Table 14: Literature Review on Catecholamines in Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Subrahmanyam S/ 1959	Women/ N=152 (toxemic pregnant women=107, normal pregnant women=25 and normal women=20	24 hrs urinary E and NE in third trimester	Increase of NE and E excretion in toxemic patients of 68.3 ± 1.71 µg and 10.4 ± 0.46, respectively was seen compared to normal pregnant women (36.2 ± 1.18 µg and 6.5 ± 0.37, respectively) and normal women (33.7 ± 1.62 µg and 5.7 ± 0.54, respectively).
O'Boyle A/ 1973	Women/ N= 91 (normal pregnant women=12, toxaemic pregnant women=67, normal non-pregnant women=12	Urinary E and NE (24 hr or sample?)	No significant change in excretion of catecholamines or in their rate of metabolism (as indicated by the vanilmandelic acid (VMA)/NE+E ratio) is evident in the 2nd or 3rd trimester when compared to non-pregnant women. Toxaemic women show a significant increase in NE and E excretion as compared to 3rd trimester pregnant women ($p<0.001$).
Castren O/ 1965	Pregnant women/ N=230 (normal pregnancy=130 and toxemia of pregnancy=100)	Urinary E and NE (24 hr? or samples?)	No clear difference in excretion of NE and E compared with non-pregnant women.

Table 14: Literature Review on Catecholamines in Pregnant Population

Author/ Year	Population/ Sample size	Measurement	Results
Hobel CJ/ 1996	Pregnant women/ N=13	Urine samples E, NE, DA after 40 minutes in recumbent position and 40 minutes of ambulation	Mean E increased slightly when the lateral recumbent position was compared with post-ambulation during both pre-stocking and post-stocking periods (women wore thigh-length support stockings for 1 week), this increase was not statistically significant. There was marginal increase in DA and significant increase in NE levels (p=0.006) in pre-stocking period, while post-stocking period showed DA decrease after ambulation (interaction, p=0.097) and increase in NE was not statistically significant.
Lederman RP/ 1977	Pregnant women/ N=21	Plasma E and NE	Third-trimester E and NE were similar to those of normal, non-pregnant subjects: sample collected under identical conditions. Compared to 3rd trimester levels, significant elevations of plasma E and NE were found in the three phases of labor.

Table 15: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Field T/ 2003	Pregnant women/ N=166	Urinary NE, E and DA from first morning urine samples during the 2nd trimester and post-partum	The high anxiety women when compared to low anxiety group were found to have high scores on depression and anger scales both prenatally and postnatally. The high anxiety group women had elevated NE ($p=0.05$), elevated E (not statistically significant) and low DA ($p=0.01$) levels prenatally.
Kemp VH/ 1989	Low socio-economic status pregnant women/ N=39 (high risk=19 and low risk=20)	Urinary E and NE from single morning voids between 9 am to 11 am	Significant difference between the groups in E levels (high levels in high risk group), but no group differences for NE, anxiety or social support scores. In low risk group: NE and E positively correlated ($r=0.42$, $p<0.03$); E and age positively correlated ($r = 0.41$, $p<0.04$); anxiety was negatively correlated with partner support ($r = -0.40$, $p<0.05$) and age($r = -0.43$, $p<0.03$). NE and E not significantly correlated with state anxiety (neither high nor low groups). In high risk group, significant negative correlation between NE and partner support ($r = -0.51$, $p<0.02$).
Katz VL/1995	Pregnant women/ N=11	Urinary E and NE from first morning voids and for subsequent 10 hrs at 24, 28, 32, 36 wks' gestation	Both E and NE levels were higher on workdays than non-workdays, 93% greater ($p<0.001$) and 52% ($p<0.001$), respectively. State anxiety test taken midway through work and non-work days showed a median 34% increase on work compared to non-work day. The median increase of work-related change expressed as a percent of non-work showed higher changes in E than NE during work compared with non-work periods.

Table 15: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Field T/ 2000	*Study 1:* Adolescent pregnant women/ N=238 (depressed group=138 and non-depressed group=100) *Study 2:* Study sample similar to study 1. Non-depressed group=51. Depressed group divided into depressed control=40 and depressed intervention =46.	*Study 1:* Urinary NE, E and DA from mother's first morning urine samples during the neonatal period, post-partum and 2nd day urine of infants *Study 2:* At 3 months, depressed group randomly assigned to intervention for 3 months	*Study 1:* At neonatal period, urinary E, NE and DA levels were significantly higher in mothers with depressive symptoms and infants of mothers with depressive symptoms. Significant correlation between the Beck depression inventory (BDI) scores at 6 months and elevated NE at neonatal period. *Study 2:* At 6 months: Mothers- For intervention group BDI scores were significantly lower than controls but higher than non-depressed group. Intervention group had significantly lower E and DA levels than controls and approximately similar to levels of non-depressed group. Infants- Though intervention group had higher NE, E and DA levels than controls; these values approximated those of non-depressed group suggesting normalization. At 12 months: Mother of intervention group had significantly lower BDI scores than controls, but still higher scores than non-depressed mothers.

Table 15: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Katz VL/1991	Pregnant women/ N = 25 (physician/nurse= 13 and controls =12)	24 hour urinary E and NE between 26-37 wks' gestation	For physician/nurse group, catecholamines were increased by 58% ($p<0.03$) during work periods compared with non-work periods. Catecholamines levels were also increased by 64% ($p<0.025$) over those of a working non-physician control group of similar gestational age.
Treadway RC/ 1969	Women/ N=30 (pregnant subjects=21 and non-pregnant controls=9)	24 hour urinary E and NE during third trimester and 1st or 2nd day after delivery	Increased feelings of depression in pregnant and post-partum women compared to their controls. NE was significantly lower in pregnant and post-partum women (increase in E was not statistically significant). Statistically significant correlation (0.76) found between decreased NE and increased depressive scores in pregnant women but not in post-partum or controls women.
Coussons-Read ME/ 2002	Pregnant women/ N=32 (low altitude= 15 and high altitude= 17)	24 hour urinary E, NE and DA	Women residing at high altitude tended to have higher levels of catecholamines during pregnancy than women living at lower altitude. No significant correlations between maternal age and/or education and catecholamine measures were found.
Castren O/ 1965	Pregnant women/ N=230 (normal=130 and toxemia of pregnancy =100)	Urinary E and NE (24 hr? or samples?)	Physical work increased the excretion slightly, and rest and reserpine treatment caused a significant decrease.

Table 15: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Field T/ 1999	Pregnant women/ N=26 (massage therapy=14 and relaxation therapy=12)	Urinary E, NE and DA (24 hr? or samples?)	Significant decrease in NE ($p<0.01$) for the massage group from the first to the last day of therapy. An increase in DA for massage group ($p<0.01$) and for relaxation group ($p<0.005$). Both groups reported feeling less anxious after the first session and less leg pain after the first and last session. Only the massage therapy group, however, reported reduced anxiety, improved mood, better sleep and less back pain by the last day of the study.
O'Boyle A/ 1973	Women/ N=91 (normal pregnant women=12, toxaemic pregnant women=67, and normal non-pregnant women=12	Urinary E and NE (24 hr or sample?)	Urinary VMA measured before and after several forms of exercise found VMA excretion to be higher after exercise, though this difference did not achieve statistical significance.
Shalev E/ 1985	Pregnant women/ N=24 (not in labor on fetal heart rate monitor, group A=12 and not in labor not on fetal heart rate monitor, group B=12)	Blood NE, E and DA	E and NE rose significantly in group A in comparison to group B after start of monitoring and began to fall after monitoring was cut off at 30 minutes. In group B there was no rise in E and NE. No difference was seen in DA levels between the two groups.

Table 15: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Vaha-Eskeli KK/ 1992	Women/ N=38 (pregnant=23 and non-pregnant=15)	Plasma E and NE	The pre-exposure values of neither of the catecholamines differed significantly between the groups. The concentration of plasma E increased significantly only in the non-pregnant group, while in the pregnant group increase in E was most in the subjects who felt discomfort during or after the thermal exposure. Plasma NE levels increased without significant differences between the groups.
Lederman RP/ 1978	Pregnant women/ N=32	Plasma NE and E	Pregnancy values in 3rd trimester were- E (39.8 +- 20 pg/ml; NE (212.4+-60.1 pg/ml). These values were not significantly different from phase 1 labor. At the onset of phase 2 of labor, self-reported anxiety and plasma E are significantly correlated. NE is significantly correlated with phase 3 labor (this maybe due to greater muscular activity and pain experienced by patients in phase 3 labor).
Kuevi V/ 1983	Healthy pregnant women/ N=44	Plasma NE and E on days 2 to 5 of the immediate post-partum period	There was a significant reduction of circulating catecholamines which correlated with mood disturbances. Women who experienced only a single day of post-partum blues had significantly lower levels of NE and E on that day compared with preceding or subsequent days.

Table 15: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Rauramo I/ 1982	Healthy pregnant women in third trimester/ N=10	Plasma NE and E before, during and after sub-maximal exercise test	Plasma concentrations of NE and E rose rapidly during the exercise, from 2.9+-0.3 to 6.9+-1.2 nmol/l and from 0.31+-0.04 to 0.47+-0.08 nmol/l respectively, and then declined rapidly thereafter. The increases in the plasma levels of NE and E showed a positive correlation with the increase in systolic blood pressure ($r=0.75$, $P<0.05$ and $r=0.75$, $P<0.05$ respectively).
Nisell H/ 1986	Pregnant women/ N=18 (pregnancy induced hypertension =9 and healthy pregnant controls=9)	Plasma NE and E before, during and after standardized mental stress test during the third trimester	At rest, NE and E levels not significantly different between the two groups. Stress caused marked increase in the feelings of being stressed and irritated in both groups (not statistically different between the groups). Stress test induced a significant increase in NE and E levels in both groups. The responses in the two groups did not differ significantly.

Table 15: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Positive studies

Author/ Year	Population/ Sample size	Measurement	Results
Giannakoulopoulos X/ 1999	Women with singleton pregnancies undergoing clinically indicated fetal blood sampling or transfusion/ N=64	Plasma NE during 2nd and 3rd trimesters	Fetal plasma NE levels were higher in samples obtained via the IHV (intra-hepatic vein which is innervated) than the PCI (placental cord insertion which is not innervated). NE concentrations increased significantly after transfusion in both the groups. The increase in the IHV transfusion group was significantly higher than the increase in the PCI transfusion group. Duration of stimulus (i.e. the time interval from puncturing the fetal trunk to collecting the last sample), but not gestational age, was associated with the rise in NE in the IHV group. Maternal NE levels rose substantially and equally after transfusion at either site. There was no significant correlation between the baselines or between the change in NE pre- and post-transfusion maternal and fetal values in either groups.

Table 16: Literature Review on Relationship of Stress and Catecholamines in Pregnant Population – Negative studies

Author/ Year	Population/ Sample size	Measurement	Results
Sanders KA/ 1997	Pregnant women/ N=10	12 hours overnight urinary E and NE	Significantly improved mood states and lower anxiety during conception cycles, but no differences in overnight catecholamine excretion within women between the conception and non-conception cycles. There was little correlation between hormonal concentrations and scores on the psychological tests during the non-conception cycles, with exception of a negative association between NE and scores on the composed anxiety ($r = - 0.34$, $p<0.05$) and clear-headed-confused ($r = - 0.37$, $p<0.05$) scales of the POMS. During conception cycle significant inverse relationship between NE and composed-anxious scale($r = - 0.52$, $p<0.05$), agreeable-hostile scale ($r = - 0.58$, $p<0.05$) and the energetic-tired scale($r = - 0.51$, $p<0.05$).
Petraglia F/ 2001	White pregnant women/ N=382	24 hr urinary E, NE and DA at 28 completed weeks of gestation	Urinary catecholamines showed a circadian pattern with a significant rise of NE and DA levels in the afternoon period and lower E levels in the night period ($p<0.05$). Low job latitude was significantly associated with a mild increase in NE and DA levels in the afternoon and night ($p<0.05$). No significant increase in catecholamine levels was found in association with the other indices of life stress or work stress, although NE values tend to be higher with most stress measures.
Hartikainen-Sorri A-L/ 1991	Pregnant women/ N=27 (normo-tensive=17 and hypertensive=10)	Plasma E and NE from 4 blood samples	The experimental noise stimulation did not induce changes in the plasma E and NE. The levels of E and NE were considerably lower in the hypertensive women.

Discrepant Results among Studies on Catecholamines and Stress

1. ***Sample Size*** - Some discrepancies remain between results of studies examining the relationship of stress and catecholamines. These might be attributed among other factors to small sample size (59, 104, 118, 153, 154). Small sample sizes may lead to reduced power with failure to detect differences even when present.
2. ***Urine Vs Blood Catecholamines*** - Some studies have measured catecholamines in 24 hour urine collection, some in samples of urine collected over varied periods of times, while others have collected blood samples to determine the catecholamines. Studies examining plasma levels of catecholamines usually tend to look at catecholamines responses to acute stressors such as labor, postpartum period, being hooked to fetal heart rate monitor, exposure to noise stimulus, thermal stress, mental stress, exercise and fetal blood transfusion. While studies hypothesizing association between catecholamines and long term stress such as anxiety, depression, work stress tend to use urine to determine catecholamine levels. This might be because catecholamines have a very short half-life and turnover or decay of circulating levels can occur within a minute or two (46, 156). Hence blood levels of catecholamines reflect acute states and are not particularly useful in studies on effects of chronic responses because of considerable fluctuations and intra-individual variability in catecholamine levels. Urinary catecholamines are excreted slowly over period of hours during which the bladder fills and therefore

provide longer, more long term measures of sympathetic nervous system activity than the blood measures (46, 156).

3. ***Psychological vs. Physical stress*** - Catecholamine responses are studied for different types of stresses such as emotional/psychological stress and physical stress. While physical stressors maybe helpful for acute catecholamine response, psychological stress might be associated mainly with chronic response. Catecholamine responses to acute and chronic stress may be different.
4. ***Confounders*** - Factors such as physical activity, drug use, consumption of alcohol and caffeinated beverages may affect catecholamines and need to be controlled for when catecholamines are used to assess stress and are linked to other factors such as adverse pregnancy outcomes (156). Not all studies described above takes these factors into consideration, which might account for varied results.
5. ***Gender***- Another important factor, "gender", might play an important role in explaining the inconsistent results found in various studies (52, 72, 74, 79, 81, 86-90). In literature, females were found to have lower excretion of urinary catecholamine as compared to men. Among the studies reviewed some studies have used females as study subjects; some studies have used only male population, while still others used both males and females. As previously mentioned researchers caution against generalizing results from studies based on men population to women population due to the gender differences.

Minimal burden of collecting urinary catecholamine levels make it a potentially useful physiological marker of stress in epidemiologic studies. By introducing feasible assessment protocols such as targeted sampling (overnight urine collection, morning/evening samples), such protocols can be introduced into large-scale epidemiological studies.

Catecholamines and Diseases

Catecholamines have also been implicated in some chronic diseases. Stress causes activation of the sympatho-adrenal system. Signals from the brain cortex are sent to the hypothalamus and via the autonomic nervous system to the adrenal medulla. This leads to increased production of all catecholamine hormones (48, 88). The short term mount in the stress hormones is favorable since it assists the mental and physical adjustment to acute environmental demands. However, the frequent and long term elevations of stress hormones may result in psychosomatic disturbances and structural changes in blood vessels leading to coronary heart disease and hypertension (48, 157). Two other factors- low control (58, 88) and inability to unwind after stressful encounters (98, 99) also play an important role in long term effects of stress hormones. Urinary NE and E were found to be lower in healthy women than in men in the age group $\leq$39 years of age (i.e. during the first half of life expectancy), while excretion was similar in men and women in the $\geq$40 years of age group (i.e. second half of life expectancy). In hypertensive individuals, catecholamine excretion was slightly higher in the first half and significantly higher in the second half of life expectancy. These differences in catecholamines excretion could contribute to

the sex-related and age-related differences in incidence of cardiovascular diseases such as hypertension and coronary heart disease (81). Resting levels of urinary catecholamines were seen to be very high in hypertensive smokers (91). Urinary NE and E excretion rates in the workplace were found to be significantly related to blood pressure (92) and to systolic blood pressure means (76), also there was a greater proportion of women with history of hypertension in the work-stressed group, $0.05<p<0.1$ (92) supporting the idea that stress in the workplace and raised catecholamines may have special significance for the prediction of cardiovascular health risk.

Froberg et al found the urinary E and NE levels to be raised along with increase in fatigue and pain in neck and back; concluding that prolonged stressful states could be detrimental to health in susceptible individuals (123). In a study on young nurses, urinary E and NE excretions were found to be higher in those reporting more frequent episodes of back pain, in addition, low control at work may have increased the activity of the sympathetic-adrenal medullary system which seemed to play an important role in the development of musculoskeletal pain (112). Urinary excretion of E, NE and DA in healthy subjects with an evening type of working capacity was 1.5, 2 and 5 times greater respectively than their excretion in persons with the morning type working capacity (158). And the evening working capacity was found 1.5 times more often among women with myocardial infarction and in men less than 44 years of age with myocardial infarction than among the corresponding healthy group. Another study on ischemic heart disease and work stress, showed that years 1945-1973

had an significantly increased cardiac deaths in West German sea pilots compared to the male population of Hamburg (159). A statistically significant rise in catecholamines was seen in volunteer pilots during three pilotage operations pointing towards increased work stress in sea pilots.

Hence, consistently high levels of catecholamines may be regarded as warning signals of long-term health consequences.

FACTORS ASSOCIATED WITH URINARY CATECHOLAMINE LEVELS IN MID-PREGNANCY

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Introduction

Stress has been associated with various conditions such as coronary heart disease and hypertension (48, 76, 81, 91, 92, 157), musculoskeletal disorders (112, 123) and preterm delivery. In United States, preterm delivery (PTD) is the leading cause of perinatal mortality and morbidity and is responsible for considerable economic costs due to short and long term sequelae .e.g. neurodevelopmental disabilities such as cerebral palsy, mental retardation and learning disabilities. Although improvement in gestational-age-related survival of preterm infants has occurred over past decades as a result of the use of antenatal corticosteroids, neonatal surfactant therapy and regionalization of perinatal care, there has been no reduction in the incidence of preterm birth (6).

The etiology of PTD remains poorly understood and until progress is made in this regard, meaningful reduction in the incidence of PTD is unlikely. Research into the causes and possible interventions to prevent PTD have important public health implications in the US. PTD is thought to have multiple pathways involving several factors. Some of the risk factors that have been explored include demographic characteristics such as ethnicity and socioeconomic status (7, 8) and biologic risk factors such as infection (9-11). During the past few decades, interest has been expressed in the potential etiologic association of psychosocial

factors, including stress, but results from previous studies have been mixed regarding the relationship between stress and PTD (12, 13, 18-30).

Stress has been measured by life events, daily hassles, perceived stress, and psychological distress (often measured by anxiety or depression). Various instruments of stress have been used in previous studies such as life experience survey, women's interview schedule for life events, social stress indicators, modified Cohen's perceived stress scale, modified life events inventory, daily hassles scale, Spielberger's state-trait anxiety inventory. Hormones such as cortisol and catecholamines (epinephrine, norepinephrine and dopamine) have been recognized as stress hormones. The positive relationship between stress and catecholamine levels in non-pregnant (17, 53, 55-58, 61-64, 67-70, 74, 76, 78, 80, 82-86, 88-90, 92, 95, 96, 98, 99, 101, 102, 105-117, 119-127) as well as pregnant population (135-138, 140, 141, 143-152) has been consistent.

Epinephrine (E) is the principal hormone of the human adrenal medulla (approximately 90%), while norepinephrine (NE) is the neurohormone of the sympathetic nerves (42, 46). Blood and urine have been used to measure the response of catecholamines to stress; but urine is the easiest source to analyze for catecholamines. Urinary catecholamines have been measured in 24-hour urine collection, in overnight collections and in samples of urine collected over varied periods of times.

Urinary catecholamines might be important in examining the relationship between stress and PTD. Before using catecholamines as biomarkers of stress to predict PTD it is essential to understand the factors associated with

catecholamines in pregnancy. This study was conducted to identify other factors that need to be considered when trying to find a link between preterm delivery and catecholamine levels. To better understand the factors associated with catecholamine levels in mid-pregnancy we conducted analysis on data from Pregnancy Outcomes and Community Health (POUCH) study.

Methods

Population:

The POUCH study is a prospective cohort study that enrolls pregnant women at 16 to 26 weeks of pregnancy from 52 participating clinics in five Michigan communities. Eligibility criteria for POUCH study include maternal age of 15 or greater, singleton pregnancy with no known chromosomal abnormality or birth defect, maternal serum alpha-fetoprotein (MSAFP) screening at 15 through 20 weeks of pregnancy, no pre-pregnancy diabetes mellitus, and proficiency in English.

Out of all eligible women approximately one third enrolled in the POUCH study. After enrollment and informed consent, POUCH participants meet with a trained study nurse and complete 'Part 1', which includes a detailed in-person interview, a self-administered questionnaire, and collection of biological samples (urine, plasma, serum, hair, vaginal smear, and vaginal fluid). Women who complete 'Part 1' are invited to participate in 'Part 2,' an at-home data collection protocol in the week following enrollment. In 'Part 2' women wear an ambulatory blood pressure monitor (ABPM) for 24 hours, complete a daily diary, and collect

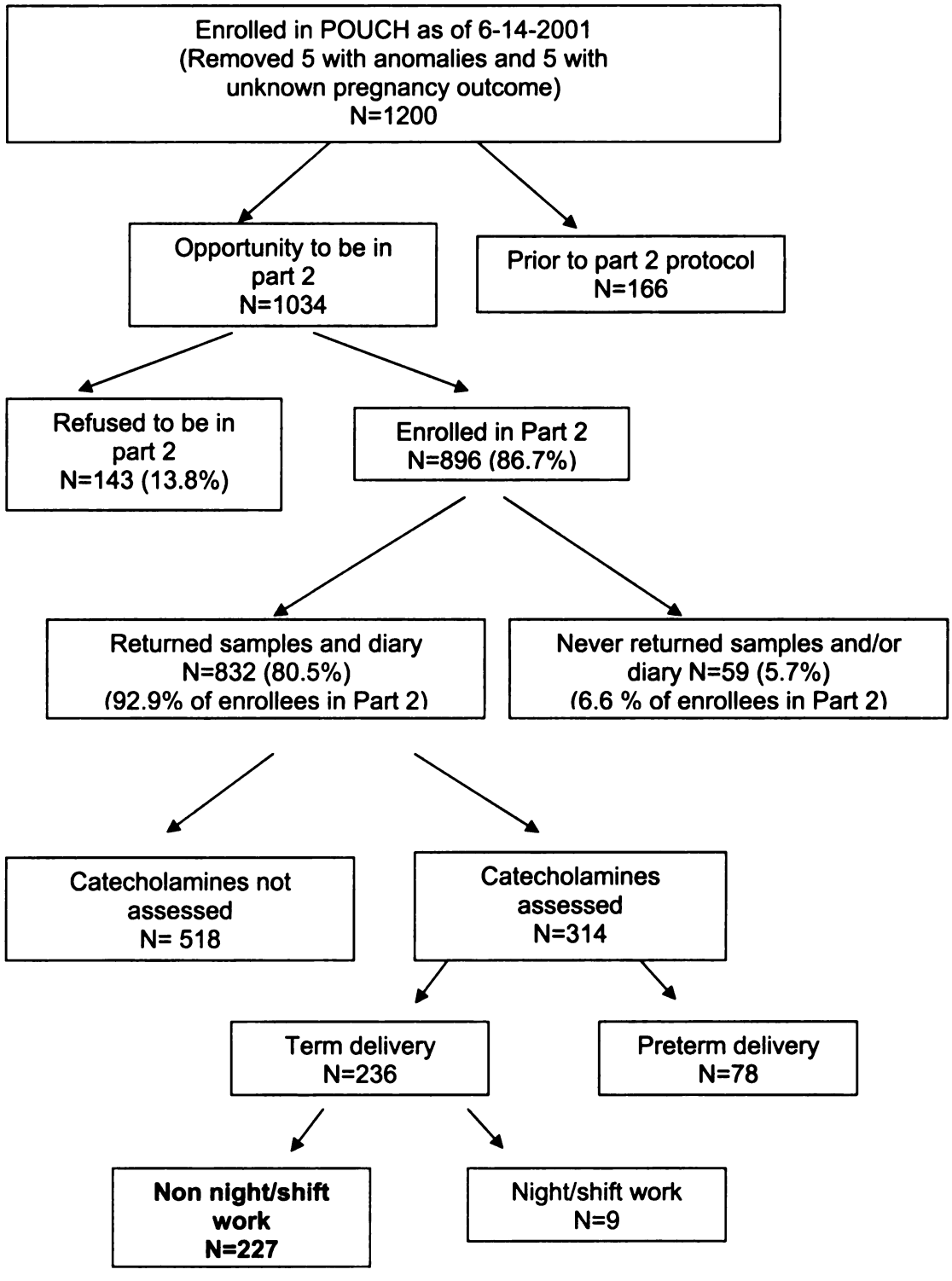
urine (10 ml) and saliva (2 ml) twice a day for three consecutive days immediately upon waking (AM) and just before bedtime (PM).

Study Sample:

This study sample comes from the first 1,210 POUCH 'Part 1' participants enrolled from September 8, 1998 through June 14, 2001. Of the 1,210 women 10 were excluded; five women were lost to follow up and five delivered an infant with a birth anomaly. The 'Part 2' protocol was initiated after 'Part 1' POUCH enrollment was underway in each community therefore only 1,034 of 1,200 women were offered the opportunity to participate in 'Part 2' during this period. Of these, 832 women (80.4%) consented and returned both samples as well as diaries (Figure 6).

Gestational age at delivery was determined by the date of delivery and by the gestational age estimated at the time of MSAFP screening, which was based on the date of the first day of the last menstrual period (LMP). LMP was used except when gestational age derived from an early ultrasound done at less than 20 weeks of gestation differed from the LMP age by 2 or more weeks, than the ultrasound age was given preference. Using a case-cohort design to conserve resources, urinary catecholamine levels were assessed in 236 women with term delivery (>= 37 weeks) and 78 women who delivered preterm (< 37 weeks). To examine factors associated with urinary catecholamine levels in pregnancy, analyses were restricted to the 227 women who delivered at term and did not work between one A.M. and eleven A.M. (i.e. no shift work) during the 'Part 2' data collection period.

Figure 6: Flowchart of study sample for catecholamine study



Catecholamine Levels in Urine:

Urinary catecholamines were measured using an extraction procedure followed by high performance liquid chromatography (HPLC) using a modified method developed by Hollenbach (160). Briefly, pH was adjusted to between 3 and 5, followed by addition of an internal standard (3, 4-dihydroxybenzylamine-hydrobromide). Extraction followed using 2M NH4OH/NH4Cl buffer and heptane containing 1% octanol. Following mixing and centrifugation, 3 ml of the organic layer was transferred into polyethylene tubes and octanol and 80mM acetic acid were added. After shaking and centrifuging, the aqueous layer was collected and stored for analysis. The HPLC system (Waters 2695 separations module) included an electrochemical detector (Waters 464). A Waters Symmetry Shield RP 4.6 x 150 mm column as used. The electrode potential was +650 mV versus an Ag/AgCl-reference electrode. The mobile phase consisted of 0.175 mMsodium decanesulfonate, 0.1 mM EDTA, 0.15 mM NaH2PO4 and 5% methanol (pH 5.1) with a flow-rate 1.2 ml/min. Catecholamines assessed were norepinephrine (NE), epinephrine (E) and dopamine (DA). Inter-assay coefficients of variations (CV) for NE, E, DA and creatinine are 10%, 14%, 10% and 8% respectively and intra-assay CV are 5%, 13%, 6% and 2% respectively.

Diary and Interview Data:

Along with collection of urine, women completed a daily diary for three consecutive days. This diary collected information on time of urine collection, time of waking and sleeping, whether it was workday or non-workday, time of starting and finishing work and Global Assessment of Recent Stress Scale

(GARS). Demographic information and data on psychosocial and behavioral factors were ascertained by in-person and self-administered interviews, which were largely composed of complete or abbreviated versions of previously validated psychosocial instruments (16).

Analytical Strategy:

During inspection of data it was found that for NE, E and DA values, approximately 5%, 27% and <0.005%, respectively were below the limit of detection (L). Non-detectable values were substituted by L/2 as recommended by Hornung et al and Finkelstein et al (161, 162). The limit of detection for NE and E was 1 nM/dl and for DA was 3 nM/dl, hence all values below 'L' for E and NE were replaced by 0.5 and for DA by 1.5.

Catecholamine values were transformed to their natural log (log nM/dl) to adjust for right skewness. The log transformed catecholamine value was divided by urine sample creatinine value to adjust for concentration of urine (log nM/dl of catecholamine per mM/L of creatinine). The creatinine adjusted, log transformed catecholamine values were used in all analyses and outliers above or below 4 standard deviations were removed. Relationships between catecholamine levels (waking, bedtime across the 3 days) were assessed using Pearson's correlations. Sample collection times were dichotomized for waking (at or before 9 AM versus after 9 AM) and bedtime (before 10 PM versus 10 PM and later), and time interval from waking to collection of sample was divided into four categories, 0-15 minutes, 16-30 minutes, 31-60 minutes, and greater than 60

minutes. This approach follows the analytic strategy used to assess factors associated with salivary cortisol levels in the same study sample (163).

Ethnicity was categorized into two groups African-American,(AFAM) and white and other, age into five groups (< 20, 20 to 24, 25 to 29, 30 to 34 and >=35), Medicaid into two (on Medicaid "yes/no"), education into three groups (< 12, =12 and >12 years), parity into three groups (0, 1, >=2) and gestational age at enrollment into 3 groups (weeks 16 to 21, 22 to 23, >23). Proc GLM was used to describe day-specific relationships between catecholamines levels, collection factors, and maternal characteristics. Repeated measures analysis by Proc Mixed was used for analyses across all 3 days. Proc mixed was chosen over proc GLM for repeated measures analysis, since proc mixed handles observations with missing values and takes into consideration within and between subject variability. All analyses were conducted using the SAS software.

Results

Maternal Characteristics:

Characteristics of the 227 women who delivered at term and are included in this analysis are described in Table 17.

Catecholamine Levels:

Table 18 shows the mean, median and standard deviation for NE, E and DA separated as waking and bedtime for 3 consecutive days.

Day-to-Day correlation coefficients for NE waking ranged from 0.37 to 0.45, NE bedtime from 0.20 to 0.44, E waking from 0.22 to 0.34, E bedtime from 0.33

to 0.40, DA waking from 0.30 to 0.33 and DA bedtime from 0.42 to 0.60 (Table 19).

Correlations for each catecholamine between waking and bedtime samples and correlations between the three urinary catecholamines are presented on Table 20.

There appeared to be only a moderate correlation for each catecholamine across three consecutive days and between their respective waking and evening values, hence it was decided to do further analysis by keeping the catecholamine values separated by day (Day 1, 2 and 3 of sample collection) and collection time (waking and bedtime). Correlations between NE and E ranged from 0.17 to 0.52, between NE and DA from 0.75 to 0.90 and between E and DA from 0.29 to 0.60. Strong correlation was seen between NE and DA but NE and DA show only a moderate correlation with E.

Relationship between Catecholamine Levels and Timing of collection, Interval from Waking to Collection of Urine sample and Work Status:

Day-specific analysis found interval from waking to collection of urine sample (p-values from 0.3-0.9), waking collection time (p-values from 0.1-0.7), bedtime collection time (p-values from 0.2-0.9), and workday/non-workday (p-values from 0.08-1.0) did not have any statistically significant relationship with any of the catecholamines. Unadjusted and adjusted repeated measures analysis across three days also did not to show any associations between catecholamines and waking collection time, bedtime collection time, and workday/non-workday. A borderline significance of E waking was seen only on day 2 with work (p=0.08)

which became non-significant when analyzed using unadjusted repeated measures model (p=0.1). In unadjusted repeated measures model, interval of collection develops a borderline significance with E waking (p=0.08), which achieves statistical significance when adjusted for waking collection time and work (p=0.04). E waking levels increase from the 0-15 minutes interval to 16-30 minutes interval, then gradually decrease over the next 2 intervals (31-60 and >60 minutes). The urine collected later than 60 minutes after waking has the lowest E waking levels as compared to the previous 3 intervals.

In a sub-analysis done only on women who work (N=123), the unadjusted repeated measures analysis across three days did not show any associations between catecholamines and interval from waking to collection of urine sample, waking collection time, and workday/non-workday. But there was a significant association seen for bedtime collection time with only NE bedtime sample (p=0.04) with collection at and after 10 pm having lower mean NE bedtime values as compared with collection before 10 pm. This association was lost (p=0.5) in the adjusted model with work status.

Relationship between Catecholamine Levels and Maternal Characteristics:

In day-specific analysis, ethnicity, Medicaid status and age were important variables associated with catecholamine levels. Ethnicity was significantly associated with all catecholamines (waking and bedtime) on all three consecutive days (p-values <0.0001 to 0.05), except E waking on day 1 (p=1.0). AFAM women had lower catecholamine values as compared to white and other. Medicaid was statistically significantly associated with NE bedtime (only on day

2, p=0.008), E waking (p-value from 0.009-0.08), E bedtime (only on day 2 and 3, p= 0.005 and 0.06, respectively), DA waking (p=0.02 only on day 2), DA bedtime (only on day 2 and 3, p=0.007 and 0.01) and not with NE waking (0.2 to 0.7).Women insured by Medicaid had lower values of all catecholamines compared to women not insured by Medicaid. The Medicaid insured groups might be confounded by ethnicity with more AFAM women insured by Medicaid. Age achieved significance for NE waking only on day 2 (p=0.07), NE bedtime only on day 2 (p=0.06), E bedtime on day 2 and 3 (p=0.03 and 0.04) and DA bedtime on day 1 and 2 (p=0.06 and 0.04).

A trend of increasing levels of urinary catecholamines was found with increasing age. Education was associated with NE waking on day 1 and 2 (p=0.03 and 0.02), NE bedtime an all 3 days (p-values ranging from 0.03 to 0.06), E waking only on day 2 (p=0.03), DA waking also only on day 2 (p=0.007) and DA bedtime on all 3 days (p-value 0.01-0.02). The group with > 12 years of education tend to have higher values for all catecholamines except for NE bedtime (where < 12 years of education had highest values on day 1 and day 3) and for E waking only on day 2 (where = 12 had highest value). The > 12 years of education group is likely to be confounded by age as women with > 35 of age will more likely be in this education group. Parity did not show any significant association with any catecholamines except on day 1 with NE waking (p=0.003) and DA waking (p=0.0006). Nulliparous women tend to have higher values particularly for NE and DA (waking as well as bedtime samples). For NE and DA, those women enrolled after 23 weeks of gestation tend to have lower values.

This might again be confounded by ethnicity, as more AFAM women tend to fall in the ">23 gestational weeks enrollment" group.

Similar means were observed for age groups 20-24 and 25-29 and 30-34 and >=35, and parity groups 1 and >= 2, hence it was decided to combined these groups.

Unadjusted repeated measures analysis using proc mixed was used to include data across 3 day (Table 21). This analysis found ethnicity to be statistically significantly related to all catecholamines except E waking, age significantly associated with all catecholamines except for NE waking and DA waking for which borderline significance was found, Medicaid was significantly associated with all catecholamines except NE waking and DA waking, education statistically significant for all catecholamines except E waking and E bedtime for which borderline significance was seen, parity was statistically significant only for NE and DA waking and border significant for DA bedtime. The trend seen in day-wise analyses remained consistent; with AFAM women, women on Medicaid and women enrolled after 23 weeks of gestation having lower catecholamine values and women >=30 years of age, having >12 years of education and nulliparous having higher catecholamine values.

All the above maternal characteristics were put into a single model to adjust for each other and results are shown in Table 22. Ethnicity and age maintained consistent association with all catecholamines except E waking with AFAM women having lower catecholamine levels as compared to white and other ethnicities and increasing catecholamine levels with increasing age groups and

women in >=30 age group having the higher catecholamine values as compared to the other two age groups (< 20 and 20 to 29). Medicaid remained significant only for E waking (p=0.05) with women insured by Medicaid having higher NE waking levels as compared to women on Medicaid and NE waking (p=0.04) with women insured by Medicaid having higher NE waking levels as compared to women not insured by Medicaid. Education was significant for NE waking (p=0.04) and DA bedtime (p=0.03) with > 12 year education women having higher values as compared to women from the other 2 groups (<12 and =12 years of education) and NE bedtime (p=0.02) with women of < 12 years education having higher values as compared to women from the other 2 groups. Parity maintained its strong significance for NE waking (p=0.002), DA waking (p<0.0001) and DA bedtime (p=0.07) with nulliparous women (parity = 0) having higher values as compared to other group women (parity >=1).

Smoking was found to be associated with NE bedtime (p=0.04) and E bedtime (p=0.05) when used as a continuous variable but not with the other catecholamine levels. When smoking was added to the above full adjusted model all maternal characteristics maintained their significance as the previous model without smoking.

In all the analyses, gestational age at enrollment was not significantly associated with any of the catecholamines. Interactions between the maternal characteristics variables could not be analyzed in the present study due to small number of women in some of the groups. The interactions will be examined when the dataset is enlarged with all POUCH participants.

Additional Preliminary Analysis:

Creatinine values were used to adjust the catecholamines values for the urine concentration. It was found that creatinine values tended to be higher in AFAM, in women insured by Medicaid and in women enrolled after 23 weeks' gestation, while creatinine values were lower in the older age group women, women with > 12 years of education and nulliparous women. It was important to assess whether this characteristic distribution of creatinine for maternal characteristics was responsible for the significant relationships seen between catecholamines and maternal characteristics. Creatinine values might vary depending on whether a woman voided during the night before she collected the morning urine sample. It is possible that these women who void during the night might have more dilute urine which could have an effect on the creatinine levels of the morning urine sample. In women who void during the night, their waking urinary catecholamine levels will reflect a shorter time period (only the early morning hours) as compared with women who do not void and urine is stored over the entire night. To answer these questions of effect of creatinine and voiding during the night, additional preliminary analyses was done on data collected on another subset of 130 women.

Collection of information on voiding during the night before collecting the waking urine sample was started after the POUCH study had began, hence this data is not available for the 227 women in the catecholamine study. Approximately 59% of the 130 women had voided during the night before collecting the waking urine sample and these women had significantly lower

creatinine as compared to the women who had not void during the night (10.35 versus 12.02 mM/L, p=0.03).

Voiding/not voiding during the night was found to be unrelated with all maternal characteristics except ethnicity. Repeated measures analysis, as described in the results section, had shown a significant association between catecholamines and ethnicity. When the variable (voided during the night "Yes/No") was added to this model, the significant relationship between ethnicity and all catecholamines waking samples remained significant. These analyses show that creatinine values and voiding might only partly explain the relationship between catecholamine and ethnicity. Hence we could infer that the relationship between catecholamines, ethnicity, age, education, Medicaid and parity maybe true and not merely a result of confounding by creatinine. This question will be reanalyzed with a larger sample size when all the POUCH "part 2" participants' data is collected and cleaned.

Table 17: Distribution of maternal characteristics in the study sample of 227 term delivered women: Ethnicity, Age, Medicaid, Education, Parity and Week of pregnancy at enrollment

Maternal Characteristics	% (N)
Ethnicity:	
African-American	29 (66)
White and other~	71 (161)
Age (yrs):	
< 20	13 (30)
20 to 24	31 (71)
25 to 29	29 (66)
30 to 34	20 (45)
>= 35	7 (15)
Medicaid:	
Yes	50 (113)
No	50 (114)
Education (yrs)*:	
< 12	18 (40)
= 12	30 (69)
> 12	52 (117)
Parity:	
0	27 (60)
1	31 (71)
>= 2	42 (96)
Week of pregnancy at enrollment:	
16 to 21	40 (91)
22 to 23	35 (80)
> 23	25 (56)

* Data missing for 1 woman
~ Ethnicity "other" is 6 % (n=14)

Table 18: Urinary catecholamine levels[+] (NE, E, DA)[#] in waking and bedtime samples on three consecutive days (N=227)

	Day 1			Day 2			Day 3		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
NE Waking	0.51	0.44	0.33	0.58	0.49	0.41	0.55	0.49	0.41
NE Bedtime	0.64	0.50	0.53	0.58	0.49	0.47	0.56	0.44	0.48
DA Waking	0.74	0.63	0.42	0.83	0.71	0.49	0.81	0.71	0.53
DA Bedtime	0.86	0.65	0.71	0.81	0.66	0.63	0.79	0.60	0.64
E Waking	0.30	0.27	0.36	0.35	0.31	0.41	0.29	0.26	0.40
E Bedtime	0.31	0.25	0.45	0.36	0.28	.50	0.33	0.26	0.47

[+] All catecholamine values expressed as log nM/dl of catecholamine per mM/L of creatinine
NE: Norepinephrine, E: Epinephrine, DA: Dopamine

Table 19: Day to Day urinary catecholamines (NE, E, DA)[#] correlation coefficients and confidence intervals around the coefficients in waking and bedtime samples (N=227)

	NE Waking r (95% CI[+])	NE Bedtime r (95% CI)	DA Waking r (95% CI)	DA Bedtime r (95% CI)	E Waking r (95% CI)	E Bedtime r (95% CI)
Day1-Day2	0.39 (0.27-0.50)	0.20 (0.07-0.32)	0.30 (0.17-0.42)	0.42 (0.30-0.52)	0.22 (0.09-0.35)	0.33 (0.20-0.44)
Day2-Day3	0.37 (0.24-0.48)	0.44 (0.33-0.55)	0.33 (0.20-0.44)	0.60 (0.50-0.68)	0.33 (0.21-0.45)	0.40 (0.28-0.50)
Day3-Day1	0.45 (0.33-0.55)	0.36 (0.24-0.47)	0.31 (0.18-0.43)	0.44 (0.32-0.54)	0.34 (0.21-0.45)	0.35 (0.23-0.46)

+ CI: Confidence Interval
# NE: Norepinephrine, E: Epinephrine, DA: Dopamine

Table 20: Correlation coefficients for urinary catecholamines between the waking and bedtime samples and between urinary catecholamines (NE, E, DA)[#] on three consecutive days (N=227)

	Day 1 r (95% CI[+])	Day 2 r (95% CI)	Day 3 r (95% CI)	Correlation averaged over 3 days
Between the waking and bedtime catecholamines:				
NE waking – NE bedtime	0.37 (0.25-0.48)	0.37 (0.24-0.48)	0.18 (0.05-0.31)	0.31
E waking – E bedtime	0.29 (0.16-0.41)	0.46 (0.35-0.56)	0.26 (0.13-0.38)	0.34
DA waking – DA bedtime	0.43 (0.32-0.54)	0.28 (0.15-0.40)	0.32 (0.20-0.44)	0.34
Between catecholamines:				
Waking				
NE – E	0.26 (0.13-0.38)	0.17 (0.03-0.29)	0.26 (0.13-0.38)	0.23
NE – DA	0.77 (0.71-0.82)	0.75 (0.69-0.80)	0.77 (0.71-0.82)	0.76
DA – E	0.30 (0.18-0.42)	0.29 (0.16-0.41)	0.35 (0.23-0.46)	0.31
Bedtime				
NE – E	0.39 (0.28-0.50)	0.52 (0.41-0.61)	0.40 (0.28-0.50)	0.44
NE – DA	0.90 (0.88-0.93)	0.88 (0.84-0.90)	0.84 (0.79-0.87)	0.87
DA – E	0.32 (0.19-0.43)	0.60 (0.51-0.68)	0.54 (0.44-0.62)	0.49

+ CI: Confidence Interval
# NE: Norepinephrine, E: Epinephrine, DA: Dopamine

Table 21: Unadjusted mean waking and bedtime urinary catecholamine[+] levels (NE, E, DA) [#]: Repeated measures analyses [†] (N=227)

	NE Waking		**NE Bedtime**		**DA Waking**		**DA Bedtime**		**E Waking**		**E Bedtime**	
	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value
Ethnicity												
White and other	0.58	0.0001**	0.66	<0.0001**	0.84	<0.0001**	0.92	<0.0001**	0.33	0.2	0.38	0.003**
AFAM	0.42		0.43		0.64		0.60		0.27		0.22	
Age												
<20	0.49	0.06*	0.57	0.003**	0.76	0.06*	0.80	0.001**	0.21	0.05**	0.23	0.008**
20 to 29	0.51		0.54		0.75		0.74		0.31		0.30	
>=30	0.61		0.74		0.87		1.04		0.37		0.45	
Medicaid												
No	0.56	0.3	0.65	0.03**	0.82	0.1	0.91	0.01**	0.37	0.002**	0.39	0.03**
Yes	0.51		0.54		0.75		0.73		0.25		0.28	

Table 21: Unadjusted mean waking and bedtime urinary catecholamine[+] levels (NE, E, DA) [#]: Repeated measures analyses [†] (N=227)

	NE Waking		**NE Bedtime**		**DA Waking**		**DA Bedtime**		**E Waking**		**E Bedtime**	
	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value
Education												
<12	0.51	0.009**	0.61	0.009**	0.71	0.02**	0.78	0.001**	0.22	0.06*	0.29	0.07*
=12	0.45		0.49		0.72		0.66		0.31		0.27	
>12	0.59		0.66		0.85		0.94		0.35		0.39	
Parity												
0	0.63	0.003**	0.64	0.3	0.93	0.0001*	0.93	0.08*	0.28	0.4	0.34	0.8
>=1	0.50		0.58		0.73		0.79		0.32		0.33	

[†] 5 different models for ethnicity, age, Medicaid, education and parity for each of the following: norepineprine waking, norepinephine bedtime, epinephrine waking, epinephrine bedime, dopamine waking and dopamine bedime

[+] All catecholamine values expressed as log nM/dl of catecholamine per mM/L of creatinine

NE: Norepinephrine, E: Epinephrine, DA: Dopamine

**p< 0.05, *p< 0.1

Table 22: Adjusted mean waking and bedtime urinary catecholamine[+] levels (NE, E, DA)[#]: Repeated measures analyses [†] (N=227)

	NE Waking		**NE Bedtime**		**DA Waking**		**DA Bedtime**		**E Waking**		**E Bedtime**	
	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value
Ethnicity												
White and other	0.59	0.0009**	0.69	0.0002**	0.87	0.001**	0.95	0.002**	0.30	0.9	0.38	0.04**
AFAM	0.44		0.48		0.69		0.69		0.30		0.26	
Age												
<20	0.45	0.08*	0.56	0.02**	0.72	0.09*	0.78	0.01**	0.25	0.5	0.22	0.06**
20 to 29	0.50		0.51		0.75		0.70		0.31		0.30	
>=30	0.59		0.68		0.87		0.96		0.33		0.43	

Table 22: Adjusted mean waking and bedtime urinary catecholamine[+] levels (NE, E, DA)[#]: Repeated measures analyses [†] (N=227)

	NE Waking		NE Bedtime		DA Waking		DA Bedtime		E Waking		E Bedtime	
	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value	Mean	P-value
Medicaid												
No	0.47	0.04**	0.56	0.5	0.74	0.1	0.79	0.6	0.35	0.05**	0.32	1.0
Yes	0.56		0.60		0.82		0.84		0.25		0.32	
Education												
<12	0.53	0.04**	0.66	0.02**	0.76	0.2	0.86	0.03**	0.27	0.7	0.35	0.5
=12	0.45		0.48		0.74		0.68		0.32		0.27	
>12	0.57		0.61		0.84		0.90		0.30		0.33	
Parity												
0	0.59	0.002**	0.61	0.3	0.89	<0.0001**	0.89	0.07*	0.29	0.8	0.35	0.3
>=1	0.44		0.55		0.67		0.74		0.30		0.29	

[†] 6 separate models, one model for each of the following: norepineprine waking, norepinephine bedtime, epinephrine waking, epinephrine bedime, dopamine waking and dopamine bedime. Each model adjusted for ethnicity, age, Medicaid, education, parity and gestational age at enrollment
[+] All catecholamine values expressed as log nM/dl of catecholamine per mM/L of creatinine
NE: Norepinephrine, E: Epinephrine, DA: Dopamine
**p<=0.05, *p< 0.1

Discussion

Studies conducted on catecholamine levels in pregnant women had sample sizes ranging from 4 to 382 women, out of which only three studies had sample sizes of more than 200 women (133, 135, 146). The present study has a fairly large sample size of 227 women, which will increase as more samples are analyzed from the POUCH study.

Most of the previous studies have collected 24 hour or overnight urine to measure the catecholamine content. A few studies do have similar protocols as the present study of collecting morning urine samples (138, 145, 146) and have been successful in supporting the hypothesis of relationships between stress and catecholamines. A study by White et al compared overnight and 24 hour urine collection to measure urinary catecholamines. They found the correlation between overnight and 24 hour urine catecholamine levels was high when both measures were standardized for urinary creatinine levels. Correlations between overnight and 24 hour urine collections for E, NE and DA were 0.37, 0.75 and 0.84 respectively. The authors support the practice of using creatinine excretion as a denominator for catecholamine excretion in community-based epidemiological studies (164). Our study uses creatinine to adjust for catecholamine excretions in urine.

Only one previous study in pregnant women (138) mentioned about NE and E being positively correlated with each other ($r=0.42$, $p<0.03$). Other studies done in non-pregnant population showed urinary E and NE to be positively correlated with range of $r=0.14$ to 0.76 (53-60). Correlation between urinary E and DA was

0.37 and between NE and DA was 0.37 to 0.77 (60, 62, 63). Intercorrelation of E, NE and DA was found to range between 0.47 to 0.69 (64). Mean urinary DA excretion pattern seems to follow mean NE excretion but not E excretion (61). Urinary E had weak correlation with the other two catecholamines (62). Our study showed a good correlation between urinary NE and DA of r=0.75 to 0.90, while fair correlations were found between NE and E and E and DA of r=0.17 to 0.52 and r=0.29 to 0.60, respectively.

The present study is unique in examining the collection factors (waking and bedtime collection, interval from waking to collection of urine sample) with respect to catecholamine levels. To our knowledge, previous studies have not examined the influences of these factors in urinary catecholamine levels. Our study shows that collection time (waking and bedtime collection time) are not associated with the levels of urinary catecholamines. In the present study, interval from waking to collection of urine sample was significantly associated with E waking after adjusting for waking urine collection time and work status. The urine collected later than 60 minutes after waking had the lowest E waking levels as compared to the previous 3 intervals. This association might be an influence of whether the woman voided or not during the night before collecting the waking urine sample.

Previous studies have consistently shown catecholamines to be higher during the work period than the non-work period. Most of the earlier studies were done in non-pregnant population (53, 55, 67-69, 76, 83, 92, 96, 98, 99, 106, 111) and only three were done in pregnant population (135, 147, 148). All these studies

collected urine to assess the catecholamine levels, either by multiple urinary samples on work and non-work day or urine samples collected over extended periods of time. Our study did not detect an association between urinary catecholamines and work/ non-work days. Since our study collects samples of urine once in morning and then in the evening the effect of the work might not be captured.

Among the maternal characteristics included in the present study, only age, education and gestational age have been examined in prior studies on pregnant population (138, 140, 141), while ethnicity, age and education have been examined in non-pregnant population (52, 57, 58, 72-76, 78-86, 98). Studies on pregnant and non-pregnant women have found no relationship between catecholamines and education and gestational age (140, 141). However in our study education was significantly related to waking and bedtime NE and bedtime DA . Our study does not find any consistent association between Medicaid insurance status and urinary catecholamines. But in our study parity was significantly associated with NE waking, DA waking and DA bedtime. A few studies failed to find an association between age and plasma and urinary catecholamines (52, 58, 72, 78, 140), but other studies, both in pregnant and non-pregnant population, found a weak to strong positive association between age and urinary catecholamines (57, 79-84). This could indicate that the older women were experiencing more stress with greater demands from children and other responsibilities. Two other researchers found that age was significantly negatively correlated with urinary E and NE in men in fifties and seventies (85)

and in children (86). Possibly the relation between age and catecholamines might be reversed in children and elderly persons compared to middle-aged population. Our study done on pregnant women support the findings that catecholamines tend to increase with increasing age. Previous studies about ethnicity and catecholamines were found only in non-pregnant population with no consistency in results. Three studies found no effect of ethnicity on urinary E and NE (75, 76) and plasma NE (77). Pratt et al (72) showed normotensive black children to have significantly lower levels of nocturnal urinary NE, while Ziegler et al (73) found white hypertensives had elevated plasma NE levels and blacks had normal levels. De Bellis et al found African-Americans excreted significantly greater concentration of urinary E and showed a trend for significantly greater concentrations of urinary NE over 24 hrs than Caucasian subjects (74). Our study showed that African American pregnant women had lower urinary catecholamine levels than pregnant women from other ethnicities.

There are some limitations to our present study. In our study, we did not collect urine over a period of 24 hours; instead we collected two 10 ml samples of urine twice a day (first morning and before bedtime urine samples). A 24 hour urine sample would have been ideal to collect but was not possible in the POUCH study because of the feasibility of such a collection in a study with a large population of approximately 3000 pregnant women. Women enrolled in the POUCH study included working women who might have posed a compliance problem for a 24 hour urine collection protocol. Another limitation of our study is the compliance with collecting the first morning void. Pregnant women tend to

have increased frequency of voiding and are likely to void during the night before collecting the morning urine sample. Such a urine sample will not capture the all night effect in the morning sample as would the sample in the women who do not void during the night. Influence of certain other factors on urinary catecholamines such as physical activity and consumption of alcohol, cigarettes and caffeinated beverages can not be adequately controlled for in the analyses. Though the POUCH study collects data on these factors during the in-person interview, this data is a global measure of a woman's habits rather than measures on the specific day of urine collection.

Conclusion

Previous studies on catecholamine levels have shown variability within individuals for urinary catecholamine values. Our study found day-to-day catecholamine levels to be only moderately correlated with each other and therefore multiple measures of catecholamine may be helpful to account for this intra-individual variability.

Our study did not find associations between time of urine collection (waking and bedtime) and urinary catecholamine levels. As previously mentioned our interest in examining these factors arises from another study using POUCH data to assess levels of salivary cortisol in pregnant women (163). Literature has found that urinary catecholamine levels are higher in the afternoon and lower in morning and night. Also urinary catecholamines do not have the diurnal pattern seen for salivary cortisol with early morning peaks. So it is reasonable to assume

that our urine sampling times did not coincide with the times when catecholamine levels peak (mid day).

The significant association found between interval from waking to urine collection and E waking can be examined with a larger sample size from POUCH study in future to assess whether this is an effect of voiding during the night before collecting the waking urine sample.

Though our study found no association between urinary catecholamine levels and work/non-workday, studies in the literature have found this association to exist with higher levels of catecholamines found on workdays. A possible reason why our study did not detect this association might be that our study collected samples of urine once in the morning and once in the evening, these samples might not have been able to capture the effect of the work period and maybe collection of urine sample during work or immediately after work are required.

Our study found ethnicity to have a consistent and significant association with all urinary catecholamine levels except epinephrine waking. AFAM women were found to have lower levels of urinary catecholamine than white and other ethnicities. The literature is not consistent for associations between catecholamine levels and ethnicity. One reason for lower levels of urinary catecholamine in AFAM women might be alterations in receptor sensitivity along the stress axis after exposure to prolonged stress. It is also possible that study results were due to lack of adjustment for unknown confounders in the urinary catecholamine and ethnicity relationship.

Age was also found to have a consistent and significant association with all urinary catecholamine levels except epinephrine waking in our study. The increasing urinary catecholamines levels seen with increase age might indicate that the older women were experiencing more stress with greater demands from children and other responsibilities.

Results from this study have implications for future studies and analyses of the POUCH data. Our study found urinary NE and DA to be highly correlated. In future analyses on POUCH data for urinary catecholamine levels, these two biomarkers could be used as correlated variables so as to increase the power in the analyses. Future studies might not need to measure both of these catecholamines.

The factors examined in our study in relation to urinary catecholamines are not an exhaustive list. In future analyses, other variables such as alcohol consumption and marital status on urinary catecholamines can be examined.

In addition, links between urinary catecholamines and maternal psychosocial measures such as self-report of stress, depression and history of abuse in the POUCH data could be explored as well as the links to risk of PTD, poor fetal growth and potential mediators of these outcomes such as infection, vascular problems. Our present study found ethnicity and age to be associated with urinary catecholamines and these might be important variables to control for in the analyses of preterm delivery and catecholamine levels.

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