Ingrida Mockutė

THE EFFECT OF MATERNAL THYROID FUNCTION AND EMOTIONAL STATE DURING PREGNANCY ON NEWBORN THYROID STIMULATING HORMONE CONCENTRATION AND ANTHROPOMETRIC MEASUREMENTS

Doctoral Dissertation

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CONTENT

LIST OF ABBREVIATIONS ................................................................. 5
LIST OF ORIGINAL PUBLICATIONS ............................................... 7
INTRODUCTION ................................................................................ 8
AIM AND OBJECTIVES .................................................................. 13
1. REVIEW OF LITERATURE .......................................................... 15
   1.1. Maternal thyroid function tests ............................................. 15
       1.1.1. Physiology basics on the thyroid functions regulations ...... 15
       1.1.2. Changes in TSH and FT4 values during pregnancy .......... 16
       1.1.3. Thyroid function tests and changes in different
              measurement techniques ................................................. 18
       1.1.4. Thyroperoxidase antibodies effect on thyroid axis
              hormones and pregnancy outcomes .............................. 24
       1.1.5. Thyroperoxidase antibodies effect on thyroid axis
              hormones and pregnancy outcomes .................................. 24
       1.1.6. Thyroid function screening controversies in pregnancy ...... 26
   1.2. The importance of the newborn thyroid stimulating hormone .. 27
       1.2.1. The fetal thyroid development and function ................. 27
       1.2.2. Iodine deficiency assessment by the newborn thyroid
              stimulating hormone .................................................... 28
       1.2.3. The newborn thyroid stimulating hormone concentrations
              in relation to maternal age and gestation at birth .......... 31
       1.2.4. Influence of delivery factors on newborn blood spot TSH
              concentrations .............................................................. 32
   1.3. Maternal psychological factors and thyroid function tests
       during pregnancy interference with newborn TSH and
       anthropometric parameters .................................................. 33
       1.3.1. Maternal thyroid axis parameters repercussions with
              newborn TSH ............................................................... 33
       1.3.2. Antenatal maternal mental state and thyroid axis function
              interference with anthropometric characteristics of the newborn 34

2. MATERIAL AND METHODS ......................................................... 38
   2.1. Study population ................................................................. 38
   2.2. Methods ............................................................................... 41
   2.3. Statistical analyses .............................................................. 44

3. RESULTS .................................................................................. 48
   3.1. Maternal thyroid function tests during pregnancy (Study I) .... 48
       3.1.1. Reference intervals for thyroid testing at each trimester
              of pregnancy .............................................................. 48
### 3.1.2. Trimester changes in concentration of TSH and FT4 during pregnancy

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
</tr>
</tbody>
</table>

### 3.1.3. Effect of thyroid peroxidase antibodies on thyroid stimulating hormone reference limits

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
</tr>
</tbody>
</table>

### 3.2. The newborn thyroid stimulating hormone (Study II, III)

#### 3.2.1. Iodine deficiency assessed by the newborn TSH concentrations (Study II)

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
</tr>
</tbody>
</table>

#### 3.2.2. The newborn TSH levels in relation to maternal age, gestation, birthweight and gender (Study III)

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
</tr>
</tbody>
</table>

#### 3.2.3. The newborn TSH levels in relation to maternal thyroid function

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
</tr>
</tbody>
</table>

### 3.3. Maternal thyroid function during pregnancy in the interference with anthropometric parameters of newborn

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
</tr>
</tbody>
</table>

### 3.4. Antenatal maternal personality and mental state and anthropometric characteristics of the newborns (Study IV, V)

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
</tr>
</tbody>
</table>

### 4. DISCUSSION

#### 4.1. Longitudinally assessed maternal thyroid axis changes during pregnancy and comparisons of reference intervals

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
</tr>
</tbody>
</table>

#### 4.2. Iodine deficiency assessed by the newborn thyroid stimulating hormone concentrations

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
</tr>
</tbody>
</table>

#### 4.3. The newborn thyroid stimulating hormone levels in relation to maternal age, gestation, birth weight and gender

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>82</td>
</tr>
</tbody>
</table>

#### 4.4. Delivery mode impact on newborn thyroid stimulating hormone concentrations

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
</tr>
</tbody>
</table>

#### 4.5. Antenatal maternal mental state and anthropometric characteristics of newborns

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>86</td>
</tr>
</tbody>
</table>

### CONCLUSIONS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
</tr>
</tbody>
</table>

### SCIENTIFIC SIGNIFICANCE OF THE STUDY

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
</tr>
</tbody>
</table>

### PRACTICAL RECOMMENDATIONS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
</tr>
</tbody>
</table>

### ACKNOWLEDGEMENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
</tr>
</tbody>
</table>

### REFERENCE LIST

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
</tr>
</tbody>
</table>

### PUBLICATIONS ON THE DISSERTATION THEME

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
</tr>
</tbody>
</table>

### ANNEXES

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>129</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

AACE – American Association of Clinical Endocrinologists
AITD – Autoimmune thyroid disease
ANOVA – Analysis of variance
ATA – American Thyroid Association
BFPI – Big Five Personality Inventory
BMI – Body Mass Index
β, Beta – Standardised regression coefficient
CH – Congenital hypothyroidism
CI – Confidence Interval
DSM–III–R – The Diagnostic and Statistical Manual of Mental Disorders, third edition, revised
EDS – Edinburgh Depression Scale
ETA – European Thyroid Association
FT3 – Free triiodothyronine
FT4 – Free thyroxine
F-test – To test if two population variances are equal
hCG – Human chorionic gondotropin
HPA – Hypothalamo-pituitary-adrenal axis
ICCID – International Council for Control of Iodine Deficiency Disorders
IDD – Iodine Deficiency Disorder
IDDM – Insulin–dependent diabetes mellitus
K-S – Kolmogorov–Smirnov test
LATS – Latin American Thyroid Society
NACB – The National Association of Clinical Biochemistry
NTDs – Neural tube defects
PPSS – Axis IV criteria of the Perceived Psychosocial Stress Scale
PPT – Postpartum thyroiditis
p–value – Probability value
r – Coefficient of Correlation
R² – Coefficient of Determination
RIA – Radioimmunoassay
SD – Standard deviation
Sig. – Significance level
STAI – State Trait Anxiety Inventor
T3 – Triiodothyronine
T3RU – T3 resin uptake
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>TBG</td>
<td>Serum thyroxine binding globulin</td>
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<td>TES</td>
<td>The Endocrine Society</td>
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<tr>
<td>THBI</td>
<td>Thyroid hormone-binding index</td>
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<td>THBR</td>
<td>Thyroid hormone-binding ratio</td>
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<tr>
<td>TFT</td>
<td>Thyroid function tests</td>
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<td>TPO</td>
<td>Thyroid peroxidase</td>
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<td>TPO-Ab</td>
<td>Thyroid peroxidase antibodies</td>
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<td>TRH</td>
<td>Thyroid stimulating hormone releasing hormone</td>
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<td>TSH</td>
<td>Thyroid stimulating hormone, thyrotrophin</td>
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<tr>
<td>t-test</td>
<td>Statistical hypothesis test</td>
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<td>TTR</td>
<td>Serum transthyretin</td>
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<td>UI</td>
<td>Urinary iodine</td>
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<tr>
<td>UNICEF</td>
<td>United Nations Children’s Fund</td>
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<tr>
<td>WHO</td>
<td>The World Health Organisation</td>
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<tr>
<td>$\eta^2$</td>
<td>The degree of association</td>
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</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications that are referred to in the text by the Roman numerals I – IV.


III. Mockutė, Ingrida; Švedas, Eimantas; Raškauskienė, Nijolė; Mickuvienė, Narseta; Bunevičius, Robertas. The newborn thyroid stimulating hormone levels in relation to maternal age and gestation at birth = Naujagimio tirostimuliuojančio hormono ryšys su motinos amžiumi bei nėštumo trukme // Lietuvos akušerija ir ginekologija = Lithuanian obstetrics & gynecology 2010, t. 13, Nr. 3, p. 278–284.


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INTRODUCTION

The issue of thyroid disease and dysfunction during pregnancy has been an important research development in recent years (1-2). Pregnancy may affect the course of thyroid disorders and, conversely, thyroid diseases may affect the course of pregnancy. Moreover, thyroid disorders and their management may affect both the pregnant woman and the developing fetus (3). Additionally, certain psychological factors, such as stress, anxiety, depressive symptoms and disorder may affect the pregnant women, the course of pregnancy and subsequently the newborn (4-6). Pregnancy could be called a psychoendocrine challenge to the mother and developing fetus. Finally, pregnant women may be under the care of multiple health care professionals, including obstetricians, endocrinologists, psychiatrists, nurse midwives, family practitioners, internists solving pregnancy, thyroid and psychiatry related issues in clinical practice, providing recommendations towards a healthier and satisfied pregnancy with the primary aim of healthy mother and child.

The prevalence of hypothyroidism during pregnancy is estimated to be 0.3–0.5% for overt hypothyroidism (OH) and 2–3% for subclinical hypothyroidism (SCH) (1-2, 7). Hyperthyroidism in pregnancy prevalence ranges from 0.1% to 0.4% with Graves’ disease accounting for 85% of cases (8-9). Isolated hypothyroxinemia has been described in approximately 2% of pregnancies (10). Thyroid autoantibodies are found in 5–15% of women of the childbearing age, and chronic autoimmune thyroiditis is the main cause of hypothyroidism during pregnancy (11-14). Between 2.2% and 2.5% of women have been found to have serum thyroid stimulating hormone (TSH) levels of 6 mIU/L or greater at 15 to 18 weeks’ gestation (12, 14).

Impaired maternal thyroid hormone metabolism during pregnancy due to dietary iodine deficiency or thyroid disease can affect the outcome of pregnancy, including miscarriage, preterm birth, preeclampsia, placental abruption, breech delivery, increased fetal mortality and impaired neurological development of the child (10-12, 15-21). Thyroid hormone dysfunction also interferes with ovulation and fertility (22-24). A sufficient thyroid function is essential to ensure a healthy pregnancy and delivery. To facilitate our understanding of the pathologic processes that affect the thyroid gland during pregnancy, it is important to understand normal physiologic processes that take place in pregnant women, including changes in thyroid function tests (25). There is no data on Lithuanian women thyroid axis hormone changes throughout the pregnancy. Moreover, we need to clarify thyroid impairment early in pregnancy for timely intervention (12, 14, 26). A proper assessment
of thyroid axis hormone concentrations plays an important role evaluating thyroid function. Research studies have shown that early maternal thyroid insufficiency, even subclinical hypothyroidism (elevated thyrotrophin (TSH) with normal free thyroxine (FT4)) and isolated hypothyroxinemia (normal TSH with lower FT4) have the potential to impair fetal neurodevelopment and delay childhood neurodevelopment (27-30). Increasing attention has therefore focused on the diagnosis and treatment of maternal thyroid dysfunction during pregnancy. Measurement of TSH and FT4 provides the primary data for evaluating thyroid status, in particular cut-off values are important for the upper TSH reference limit and for the lower FT4 reference limit.

The issue of thyroid axis hormone changes during pregnancy has not been addressed in Lithuanian women. Moreover, the thyroid hormone axis reference intervals for thyroid function assessment have not been constructed for pregnant women in Lithuania. A precise thyroid function evaluation is important not only in respect of maternal and fetal wellbeing, but more so having in mind that Lithuania is still in a vulnerable state of insufficient iodine environment (31-33). Although in recent years the understanding of thyroid physiology in pregnancy has improved, the interpretation of biochemical thyroid function tests can still be difficult. The wide variability in the lower and upper euthyroid reference limits of thyroid axis related hormones is influenced by assays used to assess hormone concentrations, by demographics of the population (ethnicity, age, sex), by iodine nutrition status, concomitant diseases, as well as by physiological conditions such as starvation or pregnancy (1-2, 34). However, development of sensitive biochemical assays that measure TSH, FT4 and free tri-iodothyronine (FT3) has improved our understanding of the gestation dependent changes of these hormones. The trend of decrease in FT4 and FT3 levels and increase in TSH values with advancing pregnancy is described in textbooks and review articles (35) and (36), yet gestation-specific reference ranges are rarely provided (2).

Some investigators have found FT4 concentrations (37) and TSH (38) to fall below the limit of normal using newer assays. The 50% plasma volume expansion in pregnancy, increase in thyroid binding globulin (TBG) production, the stimulatory effect of chorionic gonadotropin (hCG) and a relative iodine deficiency mean that thyroid hormone reference interval for non-pregnant women may not be appropriate in pregnancy (39). Therefore it was proposed to consider that pregnancy should be viewed as an ‘environmental’ factor triggering thyroid machinery and inducing thyroid pathology, especially in areas with a marginally reduced dietary iodine intake. Thus, the ranges of thyroid function tests should be not only trimester–specific but
presumably also geography–specific to account for the differences in ethnicity and iodine intake (2).

The majority of thyroid function assessing studies are based on cross-sectional data, whereas longitudinal cohort studies addressing thyroid function during pregnancy are still lacking (40-41). Longitudinal in contrast to cross-sectional studies give a better indication of what occurs in thyroid axis hormone secretion during pregnancy and how reference interval for these hormones should be constructed. Therefore our study was constructed to evaluate thyroid axis changes in pregnancy by longitudinal approach.

Only five countries in Europe, namely Austria, Finland, Norway, Sweden and Switzerland had no iodine deficiency problem in 1993 but it continued to persist in all other European countries, to some degree (42). In 2004, it was estimated that 2 billion people worldwide were at risk of iodine deficiency and 20% were in the Eastern and Western Europe being both affected (42-43). While cretinism, the most extreme expression of iodine deficiency, has become very rare and even extinct in Europe, of considerably greater concern are the more subtle degrees of mental impairment associated with iodine deficiency that lead to poor school performance, reduced intellectual ability, and impaired work capacity. Nevertheless, iodine deficiency is the most preventable cause worldwide of preventable mental retardation (44).

On the basis of the national medians of urinary iodine (UI) for the 40 European countries included in the review by the World Health Organisation (WHO) in 2007, it is estimated that the populations of 19 countries have adequate iodine nutrition, 12 have mild iodine deficiency, one country experiences moderate iodine deficiency, and eight countries have insufficient data (42). Thyroid function comes into alteration when iodine intake falls below 75µg/d (45). This situation can occur in individuals in China, India, Indonesia, but also in some countries of Europe and the United States (45). It makes the 50% decrease in iodine intake found in HANES III survey in the United States worrisome (46). The same study observed a marked urinary iodine decrease in pregnant women, compared with the earlier data (47). Lithuanian National survey data presented in 1995, revealed 62% of population with UI less than 100 μg/L (42, 48). In addition, there is growing evidence that iodine deficiency has reappeared in some European countries where it was thought to have been eliminated (31). Salt iodization remains the recommended strategy for eliminating iodine deficiency as being the most cost-effective (49). In May 2002 a Special session on Children of the United Nations General Assembly (New York) endorsed the goal of iodine deficiency disorder (IDD) elimination by the year 2005, signed by Lithuanian governors as well (42). Evaluation of pregnant women was carried out only once in Lithuania between 1998 and 2000, before implementation of
mandatory salt iodisation program in the country (32, 50). As stated in WHO report (42) monitoring of national programmes is currently insufficient, especially as it relates to measuring progress towards the goal of eliminating iodine deficiency. Therefore, it is important to evaluate the current situation on iodine sufficiency in our country and the effect of the program thereafter. Neonatal thyroid stimulating hormone (TSH) as an indicator appears to be a particularly sensitive tool in the evaluation of the iodine status of a population and in the monitoring of iodine intervention programmes (51). Thus within the same study on thyroid axis study in healthy pregnant women, we analysed TSH levels of progeny, as an indicator of iodine sufficiency in the area.

There is a well-established relationship between alterations of various hormonal systems and psychiatric disorders, both in endocrine and psychiatric patients (52). It is now widely accepted that thyroid hormone continues to play a critical role in the adult brain, influencing mood and cognition, although the details remain to be investigated (53). The relation between depression and thyroid dysfunction or thyroid autoimmunity has been documented not only in psychiatric (53-54) and general population (55-57), but also in postpartum women (58-59). It was demonstrated that subclinical hyperthyroidism increases the risk of depression in early pregnancy (60), and low antenatal thyroid functioning in late pregnancy is related to postpartum depression (59). In our study, despite endocrinological thyroid function measures, maternal personality traits and emotional state were continuously monitored. Currently, the issue of mental health assessment antenatal is broadly discussed (61). There are opinions on benefits of screening for depression in pregnancy and postpartum (62). Infants of depressed mothers display delayed psychologic, cognitive, neurologic, and motor development (61, 63), as well as antenatal maternal stress and anxiety impacts adverse pregnancy outcomes (64). Assessing women for psychosocial risk factors and symptoms of distress during regular pregnancy follow-up gives the opportunity to link women with appropriate interventions for management.

Knowing, that intrauterine life is a challenging period for fetal development and an impaired intrauterine environment affects the development of future chronic diseases (65-67), birthweight could be called a marker of fetal and newborn wellbeing or health. Therefore, our study evaluated maternal psychoendocrine impact on the newborn anthropometry, since low birth weight is associated with increased risk of mortality and morbidity of infants (68) and an overweight - with increased complications in pregnancy and prediction of metabolic outcomes in later life (69). Globally, 15.5% of all births, or more than 20 million infants worldwide, are born with low birthweight. The level of low birthweight in developing countries (16.5%) is
more than double the level in developed regions (7%) (68). On the other hand, there has been a rise in the prevalence of large newborns over a few decades in many parts of the world. In 2010, around 43 million children under five were overweight. Once considered a high-income country problem, overweight and obesity are now on the rise in low- and middle-income countries. In 2008, 1.5 billion adults, 20 years and older, were overweight (70). Birthweight is a strong indicator not only of maternal health and nutritional status, but also of the newborn's chances for survival, growth, long-term health and psychosocial development (71). Understanding the importance and the global scale of the birthweight issue, we analysed possible factors accounting for the latter with the concern on maternal personality traits and emotional state during pregnancy, as well as the impact of thyroid function.
AIM AND OBJECTIVES

The aim of the research was to evaluate maternal thyroid axis hormone and emotional state changes during pregnancy, to assess newborn thyroid stimulating hormone concentration the third day after birth and determine their interrelations and the effect on pregnancy outcomes.

The objectives of the study
1. To determine trimester-specific reference intervals and evaluate the effects of thyroid autoimmunity on thyroid stimulating hormone and free thyroxine concentrations in different trimesters of pregnancy in the cohort of healthy pregnant women.
2. To determine iodine deficiency using newborn thyroid stimulating hormone screening data as an indirect method and maternal thyroid function measures.
3. To examine the relation between newborn thyroid stimulating hormone concentration and delivery outcomes and maternal thyroid function during pregnancy.
4. To evaluate the relation of maternal thyroid function, emotional state and anthropometric characteristics of the newborns.

Scientific novelty of the study

The study was designed to assess longitudinally maternal thyroid axis hormone changes during pregnancy and consecutively establish reference intervals for TSH and FT4 in this study, which could be used for diagnosing and monitoring thyroid disorders in pregnancy. Measurement of TSH and FT4 provides the primary data for evaluating thyroid status. Since, published reference interval data are limited and absolute reference limits vary, clinical laboratories frequently use only insufficiently approved reference intervals given by the manufacturers of commercially available assays. The current worldwide discussions of reliable reference intervals for thyroid function tests in pregnancy, stimulates to present our data on healthy pregnant women in Lithuania. Variables that affect the setting of TSH and FT4 reference intervals, in particular manufacturer’s methodology and iodine status, are discussed in this paper.

This study addresses the iodine sufficiency issue on the basis of newborn thyroid stimulating hormone. The effectiveness of the salt iodisation program and the achievement of the goal of IDD elimination by the year 2005, signed by the Lithuanian governors at the United Nations General Assembly (New York) is referred to in this manuscript.
Moreover, our present study demonstrates the strongest predictors altering newborn thyroid stimulating hormone and anthropometric parameters in relation to certain maternal psychological factors together with maternal thyroid axis hormones throughout different time points in pregnancy.

Finally, our longitudinally designed research study gives a better understanding of the complex maternal–fetal interaction related to the ongoing complex physiological thyroid processes and psychoemotional aspects during pregnancy.
1. REVIEW OF LITERATURE

1.1. Maternal thyroid function tests

1.1.1. Physiology basics on the thyroid functions regulations

The TSH-releasing hormone (TRH), pyroglutamyl-histadyl-prolinamide, is synthesized in anterior hypothalamic neurons and released in the region of the median eminence. Circulating down the neurohypophyseal portal plexus, TRH binds to cell membrane receptors on anterior pituitary thyrotropes and causes production and release of TSH. The number of TRH receptors is in part regulated by T3 nuclear receptor occupancy. High T3 nuclear receptor occupancy is associated with reduced TRH receptor numbers.

TSH, a glycoprotein, is composed of alpha and beta chains linked by sulfhydryl bonds. The alpha chain is common to TSH, luteinizing hormone, follicle stimulating hormone, and human chorionic gonadotropin while the beta chain is unique to each. Hormonal production of the thyroid gland is constituted of thyroxine (T4) (80%) and triiodothyronine (T3) (20%). TSH interacts with plasma membrane receptor, which leads to endocytosis of colloid followed by fusion of the endocytic vacoule with intracellular lysosomes, which leads to digestion of the contained thyroglobulin and release of bound T4 and T3. In the circulation, whole T4 originates from thyroid secretion but most of T3 (80%) is produced extrathyroidally from T4 deiodination. Conversion of T4 to T3 may be influenced by various conditions and circulating T3 is a less reliable reflection of thyroid hormone production than T4. T4 and T3 enter the circulation and reversibly bind to circulating proteins, the most important of which is TBG, an inter-alpha-globulin, as two thirds of the T4 is carried by TBG. In pregnancy, the proportion of circulating T4 carried by increased levels of TBG is even greater, in excess of 75% (23). Other thyroxine binding proteins are transthyretin (TTR) and albumin. The circulating levels of both serum albumin and TTR remain stable, with only a slight tendency to decrease near the end of gestation, mainly as a result of hemodilution due to the increased vascular pool (72). TBG has the highest affinity for the iodothyronines, thus both T4 and T3 are tightly bound (73). The unbound T4 enters cells and is deiodinated by three enzymes at one of two potential sites. Removal of carbon 5' iodine from the outer ring of T4 by type I deiodinase, forms T3 (74). There is a preferential secretion of T3 by the thyroid gland during pregnancy, under direct influence of TSH (23). Type II deiodinase increases for maintaining T3 produc-
tion in placenta, when availability of T4 decreases. Placenta also contains type III deiodinase, which converts T4 to reverse T3 and T3 to T2 (75).

Secretion of the thyroid hormones T4 and T3 is regulated by pituitary TSH. TSH secretion, in turn, is controlled through negative feedback by thyroid hormones. There is a negative log-linear relationship between serum free T4 and TSH concentrations (76). This means that very small changes in serum free T4 concentrations induce very large reciprocal changes in serum TSH concentrations (76). As a result, thyroid function is best assessed by measuring serum TSH, assuming steady state conditions in the absence of pituitary or hypothalamic disease. It follows that high TSH and low FT4 is characteristic of hypothyroidism and low TSH and high FT4 is characteristic of hyperthyroidism (77).

1.1.2. Changes in TSH and FT4 values during pregnancy

The normal thyroid function is essential for successful fetal development, maternal morbidity and well–being of the child. Complex physiologic changes take place during pregnancy, which tend together to modify the metabolism of the thyroid and have a variable impact at different time points during gestation.

Pregnancy is accompanied by alterations in the thyroid function because of the rise in human chorionic gonadotropin (hCG) and the stimulatory effects of estrogen on thyroid binding globulin (TBG) synthesis. In early gestation hCG has two antagonistic effects on thyroid economy, tending to lower FT4 after the rapid increase in serum TBG, and to increase FT4 through its specific thyroid-stimulating action (45). The structural homology between hCG and TSH molecules has a direct stimulatory effect of hCG on thyrocytes. Respectively, that induces a small and transient increase in free thyroxine levels near the end of the 1st trimester (peak circulating hCG) and, in turn, a partial TSH suppression during normal pregnancy (23, 78). The rise in serum FT4 is proportional to peak hCG values, subsequently TSH nadir mirrors the rise of hCG concentrations (78). The peak rise in hCG and the nadir in serum TSH occur together at about 10-12 weeks of gestation. During the first trimester in approximately 20 % of healthy pregnant women, serum TSH values may be transiently lowered to subnormal values (23, 78-81). Serum TSH levels return progressively to the normal range, starting in the second trimester (82). Thereas, free thyroid hormone concentrations start decreasing during the second and third pregnancy trimesters (23, 83).

The increase in total serum T4 and T3 concentrations in the first trimester of pregnancy rise to levels about 1.5 fold those of nonpregnant women that
occurs due to an increase in serum TBG concentrations (45). Due to stimulatory estrogen effects, TBG production in liver increases, and also due to reduced plasma clearance of TBG (84), stabilization of TBG molecule, prolonged half-life due to salylation processes (23), consequently, TBG concentrations start to increase early in pregnancy and, by 16–20 weeks of gestation, have doubled (23, 78). In order to maintain unaltered FT4 levels, increase in TBG secretion is followed by increasing synthesis and diminished degradation of total T4, until a new homeostasis is reached. This is achieved during pregnancy by ~50% increase in thyroid hormone production (3, 85). The requirement for increased total T4 secretion, increases iodine requirements during pregnancy (45). In addition to an increase in serum TBG, the modest decreases in both TTR and albumin are commonly found in pregnancy, but the physiological impact of these changes is unknown (86).

In regions with the iodine supply borderline or low, the situation is clearly different, thus significant changes occur during pregnancy (23, 78, 87). In iodine sufficient conditions, the physiologic FT4 decrement that is observed during the second and third trimester remains minimal (~10%) (88), whereas it is, enhanced (~20–25%) in iodine deficient nutritional conditions (78). Iodine insufficiency revealed by pregnancy explains the progressive increase in serum TSH observed after 16 weeks of gestation (23).

The physiological changes that take place in maternal thyroid economy lead to an increase in thyroid hormone production of ~50% above preconception baseline hormone production. Moreover, renal iodide clearance augments significantly due to an increased glomerular filtration rate in the first weeks of gestation and persists thereafter (23, 45). Furthermore, iodine deprivation for the mother continues later in gestation from the passage of a part of the available iodine from maternal circulation to the fetal–placental unit (89). Hence, when iodine deprivation exists during the first half of gestation, it tends to become more severe in the final stages (3). A limited availability of iodine during pregnancy presents an additional challenge to the thyroid gland when hormone requirements are increased. In order to achieve the necessary increment in thyroid hormone production, the iodine intake needs to be increased during early pregnancy. In addition, maternal iodine intake must be increased to supply the requirements of the fetal thyroid during the second and third trimesters (45).

In the nonpregnant condition an adequate iodine intake is estimated to be 100–150 μg/d (23). When women with an iodine intake of <100 μg/d become pregnant, the pregnancies are frequently associated with thyroid function abnormalities, mainly maternal hypothyroxinemia, resulting in excessive thyroidal stimulation and goiter formation in both the mother and the child (90-94). Thus, WHO Technical Consultation held in 2005 proposed
the daily recommended nutrient intake (RNI) for iodine during pregnancy and breastfeeding should range between 200 and 300 μg/d, with an average of 250 μg/d (95). Moreover, when salt restriction is prescribed, it is highly recommended to monitor serum TSH changes and provide supplements of iodine during pregnancy (94). Furthermore, a recent study showed that in addition to consumption of iodised salt, pregnant women should take iodine supplements throughout pregnancy (96). It was proposed to consider that pregnancy should be viewed as an ‘environmental’ factor triggering the thyroid machinery and, in turn, inducing thyroid pathology in areas with a marginally reduced iodine intake (3). In clinical practice, iodine restriction is presented by relative hypothyroxinemia (T4/TBG), an elevated total T3/T4 molar ratio, and an increase in serum thyroglobulin and progressively increasing TSH levels, after nadir in the first trimester (3).

1.1.3. Thyroid function tests and changes in different measurement techniques

Nowadays, when the sensitivity and specificity of TSH assays have improved, it is recognised that the serum TSH measurement offers better sensitivity for detecting thyroid dysfunction compared to FT4 testing (97).

Variables such as age, gender, race, and season, phase of menstrual cycle, cigarette smoking, exercise, fasting or phlebotomy-induced stasis have minor effects on the reference intervals for thyroid tests in adult outpatient (2, 98-99). Thus recently, an approach to adopt age, gender, and ethnicity specific thyrotropin reference limits in population was proposed by United States researchers (100).

Serum TSH concentration is determined by immunoassay methodology. Depending on the type of label attached to the TSH antibody, the assay is variously called immunoradiometric, immunofluorimetric, immunochemiluminometric, or immunoenzymometric. Published sensitivities of the various assays range from 0.004 to 0.6 mIU/L (101).

First generation TSH radioimmunoassays had detection limits of about 1 mIU/L. Since the normal range for serum TSH is about 0.4 to 5.0 mIU/L, these assays were useful for the diagnosis of primary hypothyroidism (as serum TSH concentrations are appropriately elevated), but were not sufficiently sensitive to distinguish between normal serum TSH concentrations and the low serum TSH concentrations present in most patients with hyperthyroidism.

Second generation TSH immunometric assays have detection limits of about 0.1 mIU/L. These assays can be used as screening tests to distinguish hyperthyroidism from euthyroidism and hypothyroidism (102). However,
since the range of subnormal TSH measurement is very limited, values near
or at the detection limit do not distinguish the degree of hyperthyroidism,
and poor quality control in many laboratories can lead to erroneous values
(103).

Third generation TSH chemiluminometric assays, currently in wide use,
have detection limits of about 0.01 mIU/L. They can therefore provide
detectable TSH measurements even in mild hyperthyroidism (104). In order to
detect reliable values of serum TSH in the hyperthyroid range, one needs a
third generation assay with a functional sensitivity of at least \( \leq 0.05 \) mIU/L
(105).

TSH assay characteristics depend on the reagents, protocols, and technical
performance of the assay (106). High-quality laboratories should have
intraassay variation of less than 5% for TSH in the range of 1.0–4.5
mIU/Liter. Fewer data are available concerning interassay variation in TSH
concentration performed on the same sample; still it should be less than
10% (105, 107-109). Thus, in appropriately calibrated assays, a single TSH
determination should reasonably reflect the TSH concentration in that sam-
ple (77).

There is a linear inverse relationship between FT4 and log-linear of the
serum TSH, making the serum TSH concentration a very sensitive indicator
of the thyroid state in patients with an intact hypothalamic-pituitary axis
(45, 108). Mildly elevated serum TSH concentrations in euthyroid individu-
als are caused by circulating TSH variants of decreased biological potency
(110) and TSH resistance syndromes (111-113) or in a subclinical hypo thy-
roidism. Spurious TSH elevations due to assay interference can also occur
with circulating heterophilic antibodies (114-116) or antimouse IgG (117-
118).

Serum total T4 is usually measured by radioimmunoassay (RIA),
chemiluminometric assay, or similar immunometric technique. Most of se-
rum T4 is bound to TBG, transthyretin (also called thyroxine-binding preal-
bumin), or albumin. Serum total T4 assays measure both bound and un-
bound ("free") T4. Normal ranges vary among laboratories; a typical range
is 4.6 to 11.2 μg/dL (60 to 145 nmol/L) (98). Serum T3 is also measured by
RIA, chemiluminometric assay or other immunometric assay. T3 is less
tightly bound to TBG and transthyretin, but more tightly bound to albumin
than T4. The normal range is even more variable among laboratories than
for total T4; a typical range is approximately 75 to 195 ng/dL (1.1 to 3
nmol/L) (98).

Four different tests have been used to estimate the free T4: equilibrium
dialysis, "direct" free hormone measurements, calculating the free hormone
index by using the thyroid hormone-binding ratio or index (THBR or THBI)
via measurement of the T3 resin uptake (T3RU), and calculating the ratio of total thyroid hormone to TBG levels (119). Because none of these methods measure FT4 directly, guidelines suggest that these methods should be named "free T4 estimate tests" (120).

Current approaches to free T4 measurement are vulnerable to several method-dependent artifacts: abnormal albumin binding of T4 or of the assay tracer, the inhibition of T4 binding to TBG by medications, and the effects of critical illness, especially in heparin-treated patients, pregnancy, and the abnormalities in sick premature infants (98). Because of systematic variation between methods (whether a technique is albumin dependent or prone to incubation or dilution artifacts), it is essential to consider methodological details when evaluating free T4 estimates in aforementioned situations (121).

Equilibrium dialysis/RIA is considered as the reference methods for free thyroid hormone measurements (122-123). Routine clinical laboratories use automated direct two-step or one-step immunoassays with a high molecular weight ligand or labelled antibody (124). Nevertheless, FT4 measurements are still vulnerable to method-dependent artefacts in particular population such as patients with renal failure and pregnant women (124). In general, two-step labeled hormone back titration methods are less subjected to artefacts due to abnormal binding proteins, changes in albumin, TBG than one-step hormone analogue methods (125).

Free T4 assays frequently fail to meet performance standards in pregnant patients (126), especially when they are performed using one-step procedure (45). To compensate this, some kits have provided different normal ranges for pregnant patients, usually lower than those of non-pregnant patients. It has been suggested that total T4 measurements are more reliable during pregnancy, but the normal pregnant range for T4 is higher than that of non-pregnant patients due to TBG excess. Total T4 levels during pregnancy are 1.5-fold higher than in non-pregnant women (83). Serum TSH concentrations are not subjected to these measurement difficulties.

The T3RU is used as an indirect measure of serum thyroid hormone binding capacity, and the FT4 index (FT4I), derived from the T4 and T3RU, for correct estimates of T4 for serum binding abnormalities (101). The FT4 index based on the T3RU test shows only small fluctuations in pregnancy, while the index based on the T4/TBG ratio shows significantly lower values than those found in nonpregnant women (23).

There are narrow individual variations in thyroid hormone test values, with data suggesting that each individual has a genetically determined FT4 set-point (108). When thyroid status is stable and hypothalamic-pituitary function is intact, serum TSH measurement is more sensitive than FT4 for
detecting mild (subclinical) thyroid hormone excess or deficiency in relation to an individual’s genetic free T4 set-point (77). Serum FT4 measurement is a more reliable indicator of thyroid status than TSH when thyroid status is unstable (77).

1.1.4. Reference interval for thyroid function tests in pregnancy

Presently, there is a controversy over the type of thyroid hormone measurement that represents the most reliable interval to differentiate normal thyroid function from abnormalities associated with subtle thyroid dysfunction during pregnancy (3).

A topic of active debate rose when improvements in serum TSH assays led to better a definition of the lower limit of the reference range, for a healthy population (127). Most laboratories have used upper limit of normal for serum TSH of about 4.5 to 5.0 mIU/L. Guidelines proposed by the National Association of Clinical Biochemistry (NACB) have stated that in the future the upper limit of the serum TSH euthyroid reference range is likely to be reduced to 2.5 mIU/L for all adults, because more than 95% of screened normal euthyroid volunteers had serum TSH values between 0.4 and 2.5 mIU/L (120). However, a population study in Germany, which excluded patients with a positive family history, goiter, nodules, or positive TPO-Ab antibodies, found a normal reference range from 0.3 to 3.63 mIU/L (128). Previous studies in America have estimated that lowering the threshold from 4.5 to 2.5 mIU/L would identify an additional 9.7% patients, representing 20.6 million, with subclinical hypothyroid if the upper TSH limit decreased; the majority of them do not have thyroid disease (106).

Presently, a controversy exists as whether patients with serum TSH values between 5 to 10 mIU/L require treatment. For individuals with a serum TSH of 5–10 mIU/L, there is an increased risk of progression to overt hypothyroidism. The risk (129) is estimated to be 2.6% per year in the absence of TPO-Ab and 4.3% per year in TPO-Ab presence. After 20 years, overt hypothyroidism developed in 33% of patients with mildly raised TSH and in 55% of similar patients who also had TPO-Ab (129). Limited history data are available for individuals with serum TSH between 2.5 and 5.0 mIU/L. Surks et al. (106) obtained data for rates of progression to overt hypothyroidism for such patients from the Whickham study. When serum TSH was between 3.0 and 5.0 mIU/Liter in adults 20–40 years of age, the probability of developing hypothyroidism in 20 years was less than 10%, whereas when TPO-Ab were present, the prevalence in 20 years, increased to 15–30%.

However, the American Association of Clinical Endocrinologists (AACE), the American Thyroid Association (ATA) and Endocrine Society
(TES) (1) consensus panel has continued to recommend that 4.5 mIU/L be maintained as the upper limit of normal, reasoning that although some individuals within the range of 2.6–4.5 mIU/L may have subclinical thyroid disease, there is a lack of evidence of adverse outcome in this group (130).

Pregnancy-related thyroid function changes lately rose debates on proper evaluation of thyroid axis hormones throughout pregnancy (2). NACB recommends that ‘trimester-specific reference intervals should be used when reporting thyroid test values in pregnant women (120). Though, it has been suggested that until trimester-specific and method-specific reference ranges are established, an upper limit for TSH in pregnant women of 2.5 mIU/L (compared with 4.0–4.5 mIU/L in non-pregnant women) should be adopted (131). For women with clinical hypothyroidism anticipating pregnancy, new guidelines from TES recommend that optimal preconception levels of TSH should be <2.5 IU/L (1).

NACB states that initiation of levothyroxine (L-T4) therapy should be considered if the serum TSH level is >4.0mIU/L in the first trimester of pregnancy (77). They further recommend that if overt hypothyroidism is diagnosed during pregnancy, thyroxine doses should be rapidly titrated to reach and thereafter maintain serum TSH concentrations of <2.5 mIU/Liter in the first trimester (or 3 mIU/liter in second and third trimesters) or to trimester-specific normal TSH ranges (of <2.3, <3.1, and <3.5 for the first, second, and third trimesters, respectively) (1). Although routine screening was not recommended, the earlier AACE, ATA, TES consensus group (97) had recommended L-T4 replacement to restore serum TSH to the reference range in pregnant women found by case finding to have subclinical hypothyroidism (normal free T4 with TSH above the upper limit of normal) (97). However, it is recognized that the supporting evidence is insufficient, and such recommendations are largely based on expert consensus (132).

If one uses total T4 to estimate thyroid function, it is therefore reasonable to adapt the non pregnant reference range by multiplying this range by 1.5 starting from second half of the pregnancy (133-134).

The reference ranges provided by the manufacturers of free thyroid hormone measurement kits have been established using pools of non pregnant normal sera. Such reference ranges are no longer valid in the pregnant state because the FT4 assays are influenced by the serum changes associated with pregnancy. Therefore, it has been suggested to adapt serum FT4 reference ranges to ‘laboratory–specific’ or ‘trimester–specific’ ranges for specific use during pregnancy but, so far, no consensus has been reached worldwide on such ‘pregnancy–adapted’ ranges, and it is recommended to remain cautious in the interpretation of serum FT4 levels in pregnancy.
Figure 1.1. TSH changes during pregnancy. The graph shows median values (in rectangle) versus the range of 2.5th and 97.5th percentiles (in oval) for each trimester of pregnancy taken from eight studies of trimester-specific TSH reference intervals, reported during 2004-2009, for women without thyroid peroxidase autoantibodies, from iodine-sufficient populations. The dotted horizontal lines show the typical nonpregnant reference range (0.4–4.1 mIU/L), (Adapted from: Glinoer, Spencer, 2010 (2)).

Similarly, reference ranges should be established for serum TSH levels during pregnancy (81, 135-136). Gestation–specific reference intervals for TSH could diminish the potential risk of misinterpretation of thyroid function tests in pregnancy (81, 120).

Figure 1.1 illustrates median TSH values and the 2.5th to 97.5th serum TSH percentiles for each trimester of pregnancy, recalculated from eight studies reported between 2004 and 2009 and carried out in women negative for TPO-Ab, which were from iodine-sufficient population and confirms the downward shift of serum TSH values during pregnancy (2).

Many factors, as has been mentioned, such as ethnicity, age, manufacturer’s methodology, iodine status and rigor for selection of the reference population and calculation method may affect the establishment of reference intervals for thyroid function tests (137-138). Reference intervals may need to be gestational age specific and method specific, and other factors may also need to be taken into account. Selection of normal subjects may account for variations in reference intervals by different authors, despite the
use of the same methodology for testing (137). Several studies have attempted to derive pregnancy-specific reference ranges for thyroid function tests with inconsistent results (81, 135-136, 139-140), perhaps reflecting differences in iodine status between studies and, in some studies, the inclusion of women with thyroid autoimmunity (81, 139).

The majority of thyroid function assessing studies are based on cross-sectional data, however longitudinal studies addressing thyroid function during pregnancy are still lacking with respect to mild to moderate iodine-deficient populations (40-41). Trimester-specific intervals are needed since thyroid insufficiency may be associated with adverse obstetric outcome and fetal neuro-developmental deficits (2, 25, 134).

Moreover, accurate assessment of thyroid function during pregnancy is critical, for both the initiation of thyroid hormone therapy, and for the adjustment of thyroid hormone dose in those already receiving thyroid hormones. Knowledge about normal changes in thyroid hormone concentrations throughout pregnancy allows better individualized iodine supplementation and thus improved antenatal care. To interpret thyroid hormone tests properly reliable and population-specific reference ranges during pregnancy are needed, not least in land-locked populations where iodine intake may be insufficient. Such situations can be found in most continents, including Europe. Longitudinal, as opposed to cross-sectional data will give a better indication of what occurs in this respect in pregnancy and how reference ranges should be constructed. No relevant up to date studies have been conducted with Lithuanian pregnant women.

1.1.5. Thyroperoxidase antibodies effect on thyroid axis hormones and pregnancy outcomes

The maternal physiological changes that occur in normal pregnancy induce not only complex endocrine changes, but also an effect of the immune responses (141). Antithyroid antibodies are classified as immunoglobulin G. It is a heterogenous group of antibodies as there are antibodies against TSH-receptor, against thyroid peroxidase and also against thyroglobulin (142). Thyroid peroxidase (TPO), originally described as thyroid microsomal antigen, is present on the apical surface of thyroid follicular cells and is the antigen involved in cell-mediated cytotoxicity (143). Pregnancy is a period in which the titres of antibodies decrease to protect fetus from abortion; but just after delivery they increase again (142). The prevalence of autoimmune thyroid disease (AITD) in the pregnant population is comparable to that found in the general female population with a similar age range, i.e. between 5–15% (144).
Women who are euthyroid but carry thyroid antibodies at the onset of pregnancy have an increased risk of developing hypothyroidism during gestation (145). Forty to 60% of women with positive TPO–Ab in early pregnancy develop postpartum thyroid dysfunction, mainly postpartum thyroiditis (PPT) and Graves’ disease after delivery (142, 146). Five percent of all pregnant women and 25% of pregnant women with insulin-dependent diabetes mellitus develop PPT during the first year after delivery (147). PTT incidence is also affected by genetic influences (148), iodine intake (149), and smoking (150).

In those studies where epidemiologic information is available on groups of control women, the data show that thyroid autoimmunity is 5.2–fold more frequent in women with a diagnosis of hypothyroidism, compared with euthyroid controls (mean of 48.5% versus 9.2%). Importantly, the prevalence of thyroid antibodies in pregnant hypothyroid women depends on the severity of thyroid dysfunction (12).

Women with thyroid autoimmunity who are euthyroid in the early stages of pregnancy are at risk of developing hypothyroidism and should be monitored for elevation of TSH above the normal range (1). The risk of progression to hypothyroidism could be predicted from serum TSH levels and TPO–Ab titers measured in early pregnancy (23). When serum TSH is above 2.5 mIU/L and/or TPO–Ab titers above 1250 U/mL before 20 weeks, these markers are indicative to develop hypothyroidism by the end of pregnancy. In women with AITD, mean serum free T4 is not only significantly lower than in controls, but in addition, is at the lower limit of normality (89).

An increased risk of miscarriage, perinatal mortality, preterm delivery, placental abruption, large for gestational age infants and maternal postpartum thyroid disease have been reported in euthyroid women with positive TPO-Ab concentrations (1-4)(143, 151-154). There is an association noticed between clinical and subclinical hypothyroidism (SCH) and infertility. Female infertility was significantly associated with AITD without thyroid dysfunction (155). In a meta-analysis of four observational studies euthyroid women with positive TPO-Ab undergoing assisted reproduction technologies had two-fold higher miscarriage rates that negative for TPO-Ab (156), the same was observed by others (22, 156-157). Striking reductions in the rates of miscarriage (by 75%) and premature delivery (by 69%) were reported among women with AITD who had received thyroxine supplementation since early gestation and throughout pregnancy. Furthermore, thyroxine–treated women with AITD maintained a euthyroid status, while FT4 decreased by 30% and TSH levels increased progressively during gestation in the untreated group (4,7)(158-159). The authors concluded that the overall
risk of having a miscarriage was 3–fold to 5–fold greater in euthyroid women with AITD (154). Moreover, long-term morbidity of mothers with thyroid dysfunction or antibodies during early pregnancy seems to predict later thyroid disease and pose a risk of diabetes (160-161).

The occurrence of stressful life events (marital disharmony, housing and socioeconomic problems) and some biological factors (e.g. previous psychiatric illnesses) are strongly associated with postpartum depression. Some authors also said that postpartum depression (60, 162) and postpartum psychosis (163) depend on the presence of antithyroid antibodies during pregnancy. It is believed that cytokines which are released during the autoimmune process can affect the central nervous system and can determine changes in behaviour (142).

Moreover, recent data on the increased incidence of pregnancy loss in pregnant women with TSH levels between 2.5 and 5.0 mIU/L provides strong physiological evidence to support redefining the TSH upper limit of normal in the first trimester to 2.5 mIU/L (164). Most pregnant women are unlikely to know their antithyroid antibody status because universal screening is not routinely done. Although a positive association exists between the presence of thyroid antibodies and pregnancy loss, universal screening for antithyroid antibodies and possible treatment cannot be recommended at this time (1). Whether thyroid hormones should be given prior to or during pregnancy in euthyroid women with TPO-Ab remains controversial (165).

1.1.6. Thyroid function screening controversies in pregnancy

Establishment of thyroid axis hormone reference interval in pregnancy is important having in mind another gately debatable issue – screening for thyroid dysfunction in pregnancy. Given the prevalence and adverse outcomes associated with maternal thyroid dysfunction, currently discussion has focused on the possibility that screening pregnant women for thyroid disease could improve outcomes for both mother and child (12, 135). Currently, universal screening in asymptomatic fertile patients is not endorsed by the American College of Obstetrics and Gynecology (166), the US Preventative Task Force (167), or TES (1). In contrast, the AACE recommends that TSH be measured in women of childbearing age before pregnancy or in the first trimester (168), and ATA recommends screening beginning at the age of 35 years and then every 5 years thereafter (97). In addition, a committee of six members who had participated in a consensus statement group comprising experts from AACE, ATA, and TES (97) independently proposed a position stating that TSH testing “should be performed routinely during the pre-pregnancy evaluation or as soon as pregnancy is diagnosed” (169). Despite
these controversies, obtaining a serum TSH level for those with a history of infertility and/or recurrent miscarriage is warranted (1). Opponents of screening, however, suggest that aggressive case finding in pregnant women is appropriate for identification of maternal thyroid dysfunction during pregnancy (139, 170). Interestingly, Vaidya et al. have reported that targeted thyroid function testing of only pregnant women at high risk for thyroid disease (e.g. family history of thyroid disease) would miss about one third of women with overt and subclinical thyroid disease (171).

Whatever approach is taken to identify thyroid dysfunction in pregnant women, appropriate interpretation of thyroid function tests plays a critical role in this process. As reference intervals for thyroid function tests in pregnant women can be significantly different from those in non-pregnant women, therefore construction of trimester-specific reference intervals are essential.

1.2. The importance of the newborn thyroid stimulating hormone

1.2.1. The fetal thyroid development and function

Thyroid develops between the 2nd and 7th week of gestation. Between 8 and 11 weeks of gestation differentiation with follicle formation, concentration of iodine and formation of thyroxine occur, but full regulatory hypothalamic–pituitary–thyroid axis interactions are established between 12 and 18 weeks (172). At early gestational stages, the presence of thyroid hormones in fetal structures can only be explained by transfer of maternal thyroid hormones to the fetal compartment, because fetal production of thyroid hormones does not become efficient until mid-gestation. Thyroid hormone and specific nuclear receptors are found in fetal brain at 8 wk after conception (173). Thyroxine can be detected in amniotic fluid prior to the onset of fetal thyroid function, indicating its maternal origin by transplacental transfer (173). Between 6 and 12 weeks of gestation, if maternal total T4 concentration is set to represent 100%, the total T4 concentration in the coelomic fluid would represent 0.07% and T4 in the amniotic cavity as little as 0.0003–0.0013% of maternal total T4 concentrations (173). Fetus is solely dependent on maternal thyroxine not only during early gestation (173-174), but even after 12 weeks, thyroid hormone in fetus continues to be partly supplied by mother (175). In utero, fetal T4 is converted to reverse triiodothyronine (rT3), not active form of T3. Just before birth a switch to T3 production occurs to facilitate extrauterine survival. In normal newborns, born vaginally, there is a sharp increase in T3 and T4 levels 24–48 hours postpartum (45, 172). After delivery, the serum TSH levels in the newborn in-
creases sharply to peak at about 2 to 4 hours after birth, returning to its initial value within 48 hours (45). This neonatal TSH surge is thought to occur in delivery stress and response to rapid reduction in the environmental temperature after delivery (172). Moreover, the TSH surge is thought to contribute to the enhancement of extrathyroid conversion of T4 to T3 by D1 or D2 (176) and adrenergic stimulation of the Dio2 gene (177). Thus, maternal thyroid is extremely important source of thyroid hormones to ensure the adequate development of the fetal-maternal unit (30, 174, 178), both for trophoblast function and for normal fetal, with the concerns of neurodevelopment (174, 179). Therefore, understanding of maternal-fetal thyroid economies are also covered in our research.

1.2.2. Iodine deficiency assessment by the newborn thyroid stimulating hormone

Iodine is an essential micronutrient present in the human thyroid gland and it is an essential component of the thyroid hormones, TT4 and TT3, with iodine comprising 65% and 59% of their weights, respectively (42). The normal function of the thyroid gland, also the tendency to develop abnormalities in thyroid gland function and structure, depend greatly on the iodine intake of the subject (180-181). Iodine deficiency is one of the major global public health problems leading to endemic cretinism, goiter and mental impairment (44, 182-183).

Thyroid hormones regulate metabolic processes in most cells, play a determining role in the process of early growth and development of most organs, especially that of the brain and central nervous system in humans from the early gestation to the age of 3 years (28-29, 184-189). If iodine deficiency exists during this period and results in thyroid hormone deficiency, it might impair the development of brain and central nervous system, continuously causing an irreversible mental retardation (44). It was estimated that among the 1572 million people in the world exposed to iodine deficiency (28.9 % of the world population) 11.2 million were affected by overt cretinism, the most extreme form of mental retardation due to the deficiency and that another 43 million people were affected by some degree of mental impairment (174). Moreover, over 285 million school-age children worldwide are iodine deficient (190).

An important epidemiological consideration is that the risk of iodine deprivation during pregnancy needs to be assessed locally and closely monitored over time, because mild to moderate iodine deficiency may occur in areas that are not immediately recognized as iodine-deficient (3, 44). Another epidemiological concept should be considered that the iodine intake
may vary unexpectedly from one area to another within a given country. This occurs often in regions with mild to moderate iodine deficiency, because of significant variations in the ‘natural’ iodine content of food and water (3, 191). Lithuania, although has a small territory, the iodine sufficiency areas also vary from one region to other (32, 48, 192).

Recently it has been realized that even milder forms of iodine insufficiency could increase pregnancy loss, perinatal and infant mortality, neonatal hyperthyrotropinemia, neonatal hypothyroidism, growth retardation, and intellectual disability (193). Elevated serum TSH in the newborn indicates an insufficient supply of thyroid hormones to the developing brain (3). Congenital hypothyroidism (CH) ranges from 1 in 3000 to 1 in 4000 newborn infants (15, 194) and is a common preventable cause of mental retardation. An elevated TSH concentration in a newborn's blood is the earliest available laboratory manifestation of CH. Owing to its superior specificity and sensitivity, TSH testing is preferred over thyroxine testing (195). Neonatal thyroid screening using TSH as the primary screening test detects not only permanent sporadic CH, but also compensated or transient primary hypothyroidism, whose incidence can be as high as 1 in 10 newborns and whose main cause is iodine deficiency (51). Detection of CH in Lithuania has started in 1993, later endorsed by the Lithuanian Ministry of Health in 2005 issued regulations Nr. V–865 on management the universal newborn screening for congenital metabolic diseases (196).

The World Health Organisation (WHO), International Council for Control of Iodine Deficiency Disorders (ICCIDD) and United Nations Children’s Fund (UNICEF) suggested the use of neonatal TSH levels as one of the indicators to assess iodine deficiency as the best indicator allowing prediction of possible impairment of mental development at the population level (197). Elevated neonatal TSH is a particularly sensitive tool in the evaluation of the iodine status of a population and in the monitoring of iodine supplementation programmes (51). Though, a recent study is concerned whether newborn TSH as a tool to assess the iodine status in populations is ideal (96). Low iodine content of the thyroid of newborns follows by an accelerated turnover rate of their intrathyroidal iodine reserves. This turnover rate is 1% in adults. It is 17% in the newborn in conditions of iodine repletion, but is as high as 62% and 125% in conditions of moderate and severe iodine deficiency, respectively (51). Such an accelerated turnover rate requires thyroid hyperstimulation, consequentely followed by an increase in TSH of the newborn (51).

According to the WHO recommendation, the frequency of an elevated neonatal TSH of >5 mIU/L in whole blood (or 10 mIU/L serum) has to be less than 3% in blood samples obtained from cord or after 3 days of age and
indicates iodine sufficiency in a population (31). The frequency of newborn thyrotropin concentrations >5 mIU/L appears to be a sensitive indicator of iodine nutrition during pregnancy as well (198). Therefore, thyrotropin screening in newborns has been used to assess the severity of iodine deficiency in populations (198-201). Table 1.2.2.1 gives the indicators for assessing iodine status based on the guidelines of the WHO and ICCIDD (190, 202).

**Table 1.2.2.1. Epidemiologic criteria* for assessing severity of iodine deficiency based on whole–blood thyroid–stimulating hormone (TSH) levels**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Target</th>
<th>Severe</th>
<th>Moderate</th>
<th>Mild</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH &gt;5 mIU/L (whole blood)</td>
<td>Newborns</td>
<td>≥ 40.0%</td>
<td>20.0–39.9%</td>
<td>3.0–19.9%</td>
<td>&lt; 3.0%</td>
</tr>
</tbody>
</table>

*World Health Organization and the International Council for the Control of Iodine Deficiency Disorders (WHO/ICCIDD) (190).

The agreed and commonly accepted strategy and the method applied for the correction of iodine deficiency is universal salt iodization (USI) – the addition of suitable amounts of potassium iodide (KI), potassium iodate (KIO3) or sodium iodide to all salt for human and livestock consumption (42). Potassium salts are the most frequently used. In 2003 the governmental recommendations for use of iodized salt were introduced in Lithuania and USI program was implemented in Lithuania under the regulatory rules Nr. V–255 (50) by the order the Minister of Health of 22 April 2004. The Hygiene Norm on Hygiene for Foodstuffs was amended to include that “all food retail outlets, catering and bakery establishments shall use only iodized table salt, containing 20–40 mg/kg iodine” effective from 1 January 2005.

The Institute of Medicine recommends a daily iodine intake of 220 μg during pregnancy and 290 μg during lactation; the WHO recommends 250 μg of iodine daily for pregnant and lactating women (203). The ATA has recommended that women receive prenatal vitamins containing 150 μg of iodine daily during pregnancy and lactation (204). In practice, this requires the administration of multivitamin pills designed specifically for pregnancy purposes and containing iodine supplements (3). It should be remembered that, because of the longstanding restriction in dietary iodine before the onset of pregnancy, a lag period of approximately one trimester is inevitable before the benefits of iodine supplementation to improve thyroid function can be observed (205-206).
In 2002 iodine consumption was assessed in Lithuanian population, showing mean consumption of 120.8 μg iodine per day (women - 104.4 μg). Yet, women in cities consume just 96.7 μg iodine per day. Moreover, survey by Lithuanian National Nutrition center in 2005 revealed that only 67% of households are using iodised salt (207). Of the 40 countries reviewed by WHO in the recent report, only nine have coverage of iodized salt at the household level of at least 90% (42). According to WHO (203), Lithuania belongs to the second category countries (between 20 and 90% of households using iodised salt; median urinary iodine between 20 μg/L and 100 μg/L). Therefore, the the WHO recommended the approach of giving iodine supplement for pregnant and lactating women: as a daily oral dose of iodine as potassium iodide, so that the total iodine intake is 250μg/d of iodine, either alone or combined with other minerals and vitamins or as a single annual oral dose of 400μg of iodine as iodised oil (203).

Thefore, the aim of this study was to determine TSH levels in newborns and assess iodine deficiency issue of the implementation of USI program in Lithuania by using neonatal TSH screening data as an indirect method.

1.2.3. The newborn thyroid stimulating hormone concentrations in relation to maternal age and gestation at birth

There is known association between elevated TSH level in the newborn and brain impairment, CH and other congenital malformations predominant being cardiac, neural tube defects and dysmorphic features (208-210). From the obstetrical point of view, the risk for congenital malformations and particularly chromosomal abnormalities increases with advanced maternal age. The childbearing period in the reproductive life cycle is generally defined as between the ages of 15 and 44 years in the studies (211). Maternal age as an influencing factor on neonatal TSH levels is of particular interest, especially recent decades, when the demography of parenting is changing due to delayed decisions to motherhood (212).

Neonatal screening TSH values may vary depending on the influence of multiple methodological sample collection factors, including timing of specimen collection, the TSH assay and collection paper used (213). There are, however, limited data available on perinatal factors potentially affecting neonatal blood spot TSH levels (214-216). Whether evaluation of TSH levels in newborns is ordered as a screening test or in response to symptoms, the understanding of confounding factors has to be further explored. Moreover, it is important to identify newborn TSH levels effecting factors due to emerging studies on new screening cut-off determination (217). Therefore, our study examines the relationship between neonatal TSH levels and possi-
ble confounding factors such as maternal age, newborn birth weight, gestational age and gender. Since birth statistics over recent decades in Europe shift in favour of delaying motherhood until thirties and beyond (212), it is of interest to assess newborn TSH levels in relation to maternal age.

1.2.4. Influence of delivery factors on newborn blood spot TSH concentrations

Prior reports have indicated that consequences of labor and delivery can alter thyroid hormone function in mothers and infants at the time of delivery (218-219). Several studies have demonstrated associations between mode of delivery and measures of stress-related hormones measured in maternal blood and umbilical cord blood (220-222). In response to stress, the production of hormones including epinephrine, norepinephrine, and cortisol is increased. These increases alter the hypothalamic–pituitary–adrenal (HPA) axis, which is also involved in thyroid hormone production (223). Noradrenaline is believed to have a stimulatory influence on TSH secretion (224). The level of cord blood thyroid stimulating hormone has been found to be higher among newborns believed to have undergone greater perinatal stress, including the onset of labour (216), longer duration of second stage (225-226), nuchal encirclement of the cord (226), meconium stained amniotic fluid (225-226), caesarian section (200, 227), vacuum extraction (225), forceps extraction (228), hypoxaemia (228-229), low Apgar score (230-232), small for gestational age (229). Although, other researchers state that elevated TSH values observed in newborns delivered vaginally (216, 225-226). Vaginal deliveries requiring augmentation and cesarean sections after attempted labor are stressful for the infant, this may initiate a cascade of thyroid axis hormonal responses (216). Infants born by vaginal breech delivery also had a higher incidence of elevated levels of cord blood thyroid stimulating hormone compared with cephalic-presenting infants (233). Interestingly, the fetal presentation has been reported to have no effect on levels of newborn cord blood thyroid stimulating hormone in those born by elective caesarean section (i.e. without labour). For newborns born by emergency caesarean section after onset of labour, the median level of thyroid stimulating hormone is higher among newborns with breech presentation, compared with those with cephalic presentation (219). The median level of cord blood thyroid stimulating hormone is also higher if the cephalic presentation is a result of a prior successful external cephalic version, compared with spontaneous cephalic presentation (219). Mean TSH values were observed to be significantly lower in preterm than in full-term infants (234). The postulated mechanisms of stress-induced elevation of cord blood thyroid stimulating
hormone include catecholamine release and pituitary hyperactivity in response to intrauterine asphyxia (224). Increased differential brain perfusion and decreased thyroid perfusion during hypoxia also have been proposed (229). The mode of delivery is associated with maternal and fetal endocrine stress responses (222). Therefore, we investigated the impact of delivery mode factors on newborn the blood spot TSH results in singleton pregnancies.

1.3. Maternal psychological factors and thyroid function tests during pregnancy interference with newborn TSH and anthropometric parameters

1.3.1. Maternal thyroid axis parameters repercussions with newborn TSH

Transplacental passage of TSH from mother to fetus is negligible, but maternal T4 could be found in fetal compartment (235). Throughout gestation, the serum TSH values are greater in fetus than are present in maternal circulation and higher than would be expected in adults with normal thyroid function (45). This indicates that there is an increasing hypothalamic-pituitary resistance to T4 during fetal development which is speculated to be a consequence of increased TRH secretion (235-236). From these results it was concluded that maternal TSH does not pass through the placental barrier. In this respect, the fetal pituitary-thyroid axis functions as a unit that is essentially independent of the mother (235, 237-238).

Nevertheless, maternal thyroid dysfunction, especially in early pregnancy, may place the mother and fetus at an increased risk of adverse obstetrical outcomes (239). Untreated hypothyroidism is associated with increased risk for preeclampsia, low birth weight, placental abruption, miscarriage, and perinatal mortality (1, 12, 15, 240-242). Recently, Idris et al. found that in addition to an increased risk of low birth weight, hypothyroidism early and late in pregnancy may increase the rate of cesarean section (242). Elevated maternal serum TSH in the second trimester is also associated with an increased rate of fetal death after 16 weeks’ gestation (12). Studies have found that although women treated for hypothyroidism may have higher rates of preeclampsia (243) and cesarean section (19) than euthyroid women. Tan et al. observed that women who are treated with levothyroxine (L-T4) in pregnancy were not at increased risk of maternal or neonatal morbidity (244).

In addition to adverse obstetrical outcomes, maternal thyroid dysfunction is associated with impaired mental development of the infant (29, 239, 245). Neuropsychological deficits in the child from as early as 3 weeks to 9 years
of age have been observed. Kooistra et al. studied newborns born to mothers with serum free thyroxine (FT4) levels below the 10th percentile at 12 weeks’ gestation, and observed decreased neonatal behavioural assessment scores at three weeks of age, compared with control subjects (28). Pop et al. studied healthy infants and found that having maternal serum FT4 levels below the 10th percentile at 12 weeks’ gestation was a significant risk for impaired psychomotor development at 10 months of age (245). A similar result was observed by Kasatkina et al. (246). The finding of a low maternal serum FT4 level at five to nine weeks’ gestation correlated significantly with a lower coefficient of mental development at 6, 9, and 12 months of age (246). Mothers with low serum FT4 at 12 weeks’ gestation who continued to have low levels at weeks 24 and 32 were at risk of having a child with delays in mental and motor development at one and two years of age. This neurodevelopmental delay was even more profound when the mothers had a continuing decrease in serum FT4 as pregnancy progressed (29). Children born to women who were not treated for thyroid deficiency during pregnancy had average IQ scores at seven to nine years of age that were 7 points lower than those of controls (15).

It is clear that maternal thyroid plays an essential role in the course of pregnancy, therefore we decided to analyze newborn TSH levels at three days of age in relation to maternal thyroid hormone axis.

1.3.2. Antenatal maternal mental state and thyroid axis function interference with anthropometric characteristics of the newborn

Thyroid hormones profile changes during pregnancy (247) and may affect maternal (185, 248) as well as fetal well-being. Our understanding that thyroid disease has negative impact on pregnancy course and outcomes is increasing. Presence of thyroid antibodies in euthyroid women is associated with miscarriage (157) and during early pregnancy with maternal depression (60). It has been shown that hypothyroidism during pregnancy is associated with higher rates of gestational hypertension, fetal death, miscarriage, lower birthweight of newborns and disturbed mental development of the child (1, 12, 15, 240). It was also demonstrated that maternal hypothyroxinemia (29, 245) during pregnancy is associated with impaired mental development of the infant. However, there are scanty data on effects of changes in thyroid hormone profile during pregnancy on anthropometrics of the newborns.

The neonatal anthropometric characteristics such as birthweight are considered to be directly related to health and nutrition of mother during pregnancy and represent development of fetus. Birthweight is an important factor that determines infant and childhood morbidity and mortality (249). Mo-
reover, alterations in birth weight has been shown to be associated with a number of adverse medical outcomes later in life, such as diabetes, hypertension, metabolic and cardiovascular disorders (250-252).

Birthweight is affected to a great extent by mother’s own foetal growth and her diet from birth to pregnancy, and thus, her body composition at conception (68). Maternal anthropometric parameters, such as pregestational body size indices and gestational weight gain have repeatedly been shown to be independent determinants of the size of the child (253-254). Therefore, maternal metabolic and endocrine factors may well be independent determinants of fetal growth (255). Apart from maternal plasma glucose, studies on the role of maternal metabolic parameters as independent determinants of fetal growth are limited (255-256). Recently, in the animal models it was investigated that a low protein diet, even in the pre-implantation phase, causes vascular dysfunction in offspring resulting in high blood pressure and anxiety related behavior (257-258). Similarly, in women a high carbohydrate diet, especially when combined with low protein, suppresses placental growth which impacts on offspring birth weight (259). Furthermore, the long term diet of a woman, even from her own developing years, also has effects on her pregnancy and placental ability to support an embryo (260). It is possible that the effects of maternal anxiety, depression and stress on the developing fetus and newborn are moderated by these factors together with maternal diet (261). Studies showed that inappropriate eating behaviors are commonly followed by other psychiatric symptoms, such as anxiety and depression (262-263). Psychiatric disorders, particularly mood and anxiety disorders, have the highest prevalence in women during childbearing years (264).

Apart from well-known biomedical risk factors for fetal development, including history of adverse pregnancy events, exposure to teratogenic substances, multiple-gestations and others, various maternal psychological problems widely prevalent during pregnancy including stress, anxiety and depression were shown to have significant negative effect on fetal and neonatal outcomes (265-266) including preterm birth, obstetric complications, lower 5-minute Apgar scores, lower birthweight, congenital anomalies and postpartum depression (266-270).

The results from recent population based study showed that the prevalence of depression and/or anxiety disorders among pregnant woman is up to 30% and it seems to be higher during pregnancy than postpartum (271). In Lithuanian pregnant woman the prevalence of depressive disorders was found to be about 7% and the prevalence of symptoms of depression was reported to be about 17% (272). Depression and anxiety disorders might have a negative impact on well-being of pregnant woman, on their relationship
with family members and might interfere with fetus growth and development. If untreated, these antenatal mood disorders together with socioeconomic deprivation and with other adverse factors may increase the likelihood of postnatal depression (263, 273). Furthermore, antenatal maternal depression as well as antenatal anxiety disorder is a risk factors for behavioral and emotional problems (274) and psychotic illness (275-276) in later life of children (277).

A recent study in a large sample of newborns showed that trait anxiety in the first two trimesters of pregnancy is associated with lower birthweight and shorter birth length after controlling for confounders (278). Although an association between pathophysiology of these psychiatric disorders and pregnancy outcomes remains unclear, it is thought that depression, anxiety and stress during pregnancy might have negative effect on mother’s health related behaviors, such as increased alcohol and tobacco consumption or poor nutrition that are associated with decreased birthweight (279). Moreover, depression, anxiety and stress were showed to cause a dysregulation of HPA axis (261, 280), decreased fetal growth hormones (265) and increased uterine artery resistance (281-282). Both maternal trait anxiety and maternal experience of anxiety during pregnancy determine offspring development (283) so, importantly, the study shows that maternal anxiety impacts on offspring in an adverse way. Additionally, stress in pregnancy programs high anxiety in offspring (284). If stress is sustained (chronic), the typical response is elevated basal glucocorticoid concentrations, altered negative feedback to the HPA axis (285-286). Lately it is discussed, that in control of maternal anxiety there is an emerging role for progesterone secretion in the brain (287-288). Increasing anxiety can lead to depression, which can be very severe perinatally. Interestingly, anxiety decreases in early gestation and remains subdued until birth (289). Pregnancy anxiety has been recognized as emerging in women that generally lack social support (290) and may be living under environment-related stressful conditions, while lack of physical activity leads to higher anxiety and mood disturbances (291). It seems that chronic stress can induce maternal anxiety in rodents (292) and even predispose to postnatal depression.

The interaction between maternal personality traits during pregnancy and neonatal outcomes is not widely studied, but it is possible that some personality traits might predispose maternal behavior that interferes with well-being of fetus and that others may have protective effect. It is known that adults with high conscientiousness have better health outcomes and longevity whereas adults with high neuroticism have poorer health outcomes and more likely to engage in lifestyle risk activities, such as smoking and alcohol intake (293). High level of neuroticism is associated with higher levels
of anxiety and stressfulness (294). In pregnant women such personality traits might influence neonatal outcomes, including anthropometric characteristics (261). Personality traits may modulate emotional and endocrine responses to stress causing changes in hormonal profile that may lead to an unfavorable environment to the development of fetus (268, 295-296).

Therefore, the aim of the present study was to assess whether psychological factors and thyroid axis hormone concentrations during pregnancy predict neonatal anthropometric characteristics.
2. MATERIAL AND METHODS

2.1. Study population

Pregnant women were eligible for enrolment into the study if they presented for the antenatal care in Kaunas, Lithuania, at the Department of Obstetrics and Gynecology at Kaunas University of Medicine as well as at Šilainiai Primary Health Care Center between 2003 and 2005 years and agreed to participate giving written informed consents these women were continuously enrolled in the study: "Effect of psychoendocrine challenge during gestation and delivery to well-being of mother and child: multical study". The study and its consent procedures were approved by the Regional Committee of Ethics in Biomedical Research at the Kaunas University of Medicine, Kaunas, Lithuania (No. 84/2002 and P1-84/2002).

A total of 322 pregnant women were assigned for participation. Subjects in the different studies are shown in Figure 2.1.1. Exclusion criteria were known extragenital disease, including current or past thyroid disease, thyroid function modifying medication, family history of thyroid disease, multiple pregnancy, hyperemesis gravidarum, fetal congenital abnormalities. Attendance to all assigned serum thyroid hormone samplings and emotional state evaluation was required. Of these 322 women, 15 did not attend any serum sampling and 18 women were excluded because of type I diabetes (n=3), multiple sclerosis (n=1), pre-existing goiter (n=6), thyroid hormone therapy (n=1), toxoplasmosis during pregnancy (n=1), twin pregnancy (n=4) and fetal cerebral malformations diagnosed at ultrasound screening (n=2). An initial sample group consisted of 289 apparently healthy pregnant women (mean age 28.3 ± 4.9 years; range 18–41 years), and they were evaluated for thyroid function during the first (12th–16th week), the second (22nd–26th week) and the third trimester (32nd–36th week) of pregnancy. Of them, 105 missed at least 1 meeting of hormonal measurement, therefore excluded from the study data analysis (Figure 2.1.1). The final analysis of maternal thyroid function and thyroid immune status was performed using data from 184 pregnant women. Our research was constructed by initiation of five study designs. Main characteristics of the cohort are provided in Table 2.1.1.

The study I assessed maternal thyroid function tests during pregnancy. Of 184 pregnant women, 23 (12.5%) women were found to have elevated TPO-Ab concentrations (mean age 27 ± 5 years, range 20–40). After excluding TPO-Ab positive women, the remaining 161 (87.5%) data sets were used as
A reference population establishing reference intervals for the thyroid hormones concentrations in three trimesters of pregnancy. In this group, one woman (0.6%) had a TSH>4.5 mIU/L, and 40 (24.8%) had TSH<0.4 mIU/L in the 1st trimester.

Figure 2.1.1. General design of the study

Study II evaluated iodine insufficiency by using newborn heel blood spot TSH measurements taken on the third day after birth. There were 74 newborns excluded, due to early TSH measure. The third day dry blood spot TSH was obtained from 215 newborns. Two newborns were excluded on the basis of incorrect typing of laboratory results. In iodine study, 213 newborns were used as target group for analysis.
Table 2.1.1. Characteristics of the study women and their children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study I</th>
<th>Study III, IV, V</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age* (years)</td>
<td>N=184</td>
<td>N=173</td>
</tr>
<tr>
<td>Mean (SD), range</td>
<td>28.2 ± 4.8 (19–41)</td>
<td>28.3 ± 4.8 (19–41)</td>
</tr>
<tr>
<td>Prepregnancy weight* (kg)</td>
<td>76.6 ± 10.6</td>
<td>76.6 ± 10.6</td>
</tr>
<tr>
<td>Height* (cm)</td>
<td>167.8 ± 5.6</td>
<td>167.8 ± 5.6</td>
</tr>
<tr>
<td>Weight gain*, kg/week</td>
<td>0.40 ± 0.1</td>
<td>0.39 ± 0.2</td>
</tr>
<tr>
<td>Prepregnancy BMI*, kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>27.2 ± 3.5 (20.7–41.7)</td>
<td>27.2 ± 3.5 (20.7–41.7)</td>
</tr>
<tr>
<td>BMI&gt;30 kg/m², n (%)</td>
<td>36 (19.6)</td>
<td>34 (19.7)</td>
</tr>
<tr>
<td>Nullipara, n (%)</td>
<td>114 (62.9)</td>
<td>105 (60.7)</td>
</tr>
<tr>
<td>Average parity*</td>
<td>1.9 ± 1.1</td>
<td>1.9 ± 1.1</td>
</tr>
<tr>
<td><strong>Newborn variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (% males)</td>
<td>Study II N=213</td>
<td>N=173</td>
</tr>
<tr>
<td></td>
<td>120 (56.3)</td>
<td>96 (55.4)</td>
</tr>
<tr>
<td>Gestational age* (weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>39.6 ± 1.2 (35–41)</td>
<td>39.6 ± 1.0 (35–41)</td>
</tr>
<tr>
<td>Premature, n (%) &lt;37 weeks</td>
<td>7 (3.2)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Birthweight* (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>3.558 ± 448 (2176–4700)</td>
<td>3.550 ± 448 (2176–4650)</td>
</tr>
<tr>
<td>Birth height* (cm), (range)</td>
<td>51.8 ± 2.1 (46–63)</td>
<td>51.8 ± 2 (46–63)</td>
</tr>
</tbody>
</table>

* Characteristic described as mean±S.D.or n (%) were appropriate; BMI, body mass index

Studies III, IV, V required mother-newborn pairs data. Further data was used to examine the relation between newborn thyroid stimulating hormone concentration and delivery outcomes and maternal thyroid function during pregnancy (study III). Additionally, data was used to evaluate the relation of maternal thyroid function, emotional state and anthropometric characteristics of the newborns (study IV, V). For those studies, mother’s birth delivery medical records were reviewed for maternal and newborn delivery data, anthropometric characteristics. Delivery case data of 6 newborns was not available. Three women were excluded from the study because they had diagnosis of gestational diabetes, 2 women were excluded from the study because they delivered very low birthweight newborns (<1500 g). A total sample of 173 mother–newborn pairs was finally included in the analysis. The main characteristics of the cohort are provided in Table 2.1.1.
2.2. Methods

Assays. Blood sample analysis for thyroid function during pregnancy was carried out and serum TSH and FT₄ were measured in all trimesters of gestation. Venous blood samples for TSH and FT₄ were drawn from 9 am to 14 pm in the first trimester at 12-16, second trimester at 22-26 and third trimester at 32-36 weeks of gestation. In the first trimester we also assessed TPO-Ab concentrations. All samples were centrifuged from 14 till 16.30 pm daily. Serum was collected in tubes without gel barriers or clot-promoting additives and frozen at -70°C. Once thawed before analyses, each assay was performed on all samples using the same kits to minimize inter-assay variation at the Laboratory in Tilburg University, The Netherlands.

TSH was measured using a solid–phase, two site chemiluminescent enzyme immunometric assay (IMMULITE Third generation TSH, Diagnostic Products Corporation, Los Angeles, USA). The inter-assay coefficients of variation were 5.0 % and 4.4 % at concentrations 0.22 and 2.9 mIU/L, respectively. FT₄ concentration was measured with a solid–phase immunometric assay (IMMULITE Free T₄). The interassay coefficients of variation for this technique were 6.7 % and 4.4 % at concentrations 11.6 and 31.5 pmol/L, respectively.

The Immulite assay provided a TSH reference interval of 0.4–4.5 mIU/L and a FT4 reference interval 10.3–25.7 pmol/L for nonpregnant adults. The assay manufacturer’s population reference intervals calculated from serum specimens from individuals with normal thyroid function (iodine status wasn’t defined in the manufacturers specifications). The reference intervals for nonpregnant adults provided by the manufacturer are also included for comparison with gestational age–specific reference interval.

Finally, the IMMULITE TPO-Ab kit was used for the determination of TPO-Ab. The inter–assay coefficients of variation for this analysis were 9.0 % and 9.5 % for concentrations 40 and 526 IU/mL, respectively. The TPO-Ab assay was standardized in terms of the International Reference Preparation for TPO-Ab MRC 66/387. Reference intervals for TPO-Ab (< 35 IU/ml) were taken from the manufacturer’s kit insert. TPO-Ab titers above 35 IU/ml were defined as elevated; TPO-Ab titers below 35 IU/ml were defined as normal concentrations.

In study II, newborns were used as target groups for iodine monitoring. Blood spot TSH measures were used from the National Neonatal Screening Program for congenital hypothyroidism. Recommended timing of taking blood from a heel prick is better after 48 hours, of age to minimize the false positive high TSH due to the physiological neonatal TSH surge that elevates TSH levels and causes dynamic T4 and T3 changes in the first 1 or 2 days.
after birth (297). Therefore our blood spot TSH data was obtained on the third day after birth, according to the institutional regulations by the nurse–neonatologist. The neonatal enzyme immunoassay with fluorometric detection (Neonatal hTSH FEIA Plus, product No. 6199880) was used for determination of TSH from blood specimens dried on filter paper. The newborn Screening Programme Centre recommends that cut–off for a blood spot TSH 10 mIU/L is used to detect congenital hypothyroidism (217). All measures of TSH in blood specimens dried on filter paper were analyzed at the Centre for Medical Genetics, Vilnius University Hospital.

**Neonatal and maternal parameters (study II–V).** Detailed information on maternal and neonatal factors was obtained from medical records from three delivery centers: Kaunas Medical University Clinics, P. Mažylis Maternity Hospital, Kaunas Christian Maternity Hospital and Kaunas 2nd Clinical Hospital. Gestational age was defined by the known date of last menstrual period and/or ascertained by early ultrasound assessment. Infants delivered before 37 weeks of gestation are considered premature. Length of gestation at delivery was treated as a continuous variable measured as the number of weeks of gestation at delivery. Infant birth weight in grams was examined as a continuous variable. From birth delivery medical case histories we analyzed gender of the newborns, birth weight (in grams), height (in centimeters) and gestational age. Weight and height of the newborns were substantiated by neonatologist who supervised childbirth in the first 24 postnatal hours. Maternal prepregnancy weight (in kilograms), height (in centimeters) were measured at the first antenatal visit by observing nurse. Weight gain (in kilograms per week) was calculated out of the measured weight at delivery. We also calculated Body Mass Index (BMI) of the mother and the newborn, using formula: BMI = weight (kilograms) / height² (meters). Maternal age at delivery, delivery mode was registered.

Apgar scores on the 1st and 5th minutes of extraterine life of the newborns were evaluated by neonatologist who supervised childbirth in the 1st and 5th postpartum minutes. Apgar score is used in clinical practice to assess a newborn’s respiratory effort, heart rate, color, tone and reflex irritability (Table 2.2.1). Scoring range is from 0 to 10. Apgar scores between 8 and 10 indicate that the newborn is making smooth transition to extraterine life; scores ≤7 are associated with increased morbidity and mortality of newborns.

**Study IV, V.** Pregnant women were evaluated three times by psychiatrist (L.K.) for stress, depression symptoms and anxiety in the first trimester (12th–16th weeks), in the second trimester (22nd–26th weeks) and in the third trimester (32nd–36th weeks) of pregnancy and were evaluated for personality traits in the second trimester of pregnancy. Thesis “Depression and
its relationship to thyroid function during pregnancy” has been defended by psychiatrist (L.K.).

Table 2.2.1. Apgar scores

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>All blue, pale</td>
<td>Pink body, blue</td>
<td>All pink</td>
<td></td>
</tr>
<tr>
<td></td>
<td>extremities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>Absent</td>
<td>&lt;100 beats/min</td>
<td>&gt;100 beats/min</td>
<td></td>
</tr>
<tr>
<td>Reflex response to nasal catheter/tactile stimulation</td>
<td>None</td>
<td>Grimace</td>
<td>Sneeze, cough</td>
<td></td>
</tr>
<tr>
<td>Muscle ton</td>
<td>Limp</td>
<td>Some flexion of</td>
<td>Active</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>extremities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration</td>
<td>Absent</td>
<td>Irregular, slow</td>
<td>Good, crying</td>
<td></td>
</tr>
</tbody>
</table>

_Psychosocial stressors_ were classified according to the life events using the Diagnostic and Statistical Manual of Mental Disorders, third edition, revised (DSM–III–R) (298), Axis IV criteria of the Perceived Psychosocial Stress Scale (PPSS). This instrument evaluates acute and chronic perceived psychosocial stressors. Women were interviewed about negative life events that are considered to cause psychosocial stress. Each negative life event is rated according to how severe it would be to an average individual. The score for chronic stress and the score for acute stress are set and the higher score indicates the higher perceived chronic stress or the higher perceived acute stress. Score of 1 indicates no psychosocial stress, 2 – mild psychosocial stress, 3 – medium psychosocial stress, 4 – severe psychosocial stress, 5 – very severe psychosocial stress and 6 – catastrophic psychosocial stress. In this study we categorized women in two groups, those who did experience acute/chronic stress with PPSS score of two or more; and those who did not experience PPSS.

_Antenatal maternal personality traits_ were evaluated using Lithuanian version of the Big Five Personality Inventory (BFPI) (294). An instrument of 44 questions designed to assess the Big Five personality dimensions of Extroversion, Agreeableness, Conscientiousness, Neuroticism and Openness. BFPI consists of 5 subscales. Extroversion and neuroticism subscale consist of 8 items, agreeableness and conscientiousness of 9, openness of 10, respectively. Each item is a descriptor and is rated in a 5–point scale from 1 (agree strongly) to 5 (disagree strongly). The BFPI showed good psychometric properties (294).

The validated Lithuanian version (299) of the _Edinburgh Depression Scale_ (EDS) was used to measure severity of depressive symptoms (300).
Though originally, this instrument was designed for screening of postnatal depression, today it is widely used for evaluation of depressive symptoms throughout all periods of women’s life in clinical practice as well as in epidemiological studies (301). The EDS is beneficial against other instruments used for screening of depressive symptoms during pregnancy because it evaluates psychological, cognitive, but not physical symptoms of depression that are prevalent during pregnancy. The EDS is easy to administer and most important, it is an effective screening tool for identifying woman with depressive symptoms during pregnancy. The EDS is a ten item selfrating instrument and takes 2 to 5 minutes to complete. Each item is scored from 0 to 3, to which subject responds and is based on her experience over the past seven days. Possible scoring range is from 0 to 30. Higher score on the EDS indicates higher severity of depressive symptoms. Depression is clinically significant, when EDS score is $\geq 12$.

Symptoms of anxiety were evaluated using the Spielberger State-Trait Anxiety Inventory (STAI) (302). The STAI – Trait scale is widely used for clinical and research purposes in psychiatric population as well as in mentally healthy subjects, including pregnant women, to assess severity of anxiety symptoms (303). The Trait-Anxiety scale of STAI consists of twenty statements that assess how respondents feel "generally". We considered that women are positive for symptoms of anxiety if they scored 45 or more on the STAI-T.

Women were also interviewed about smoking and use of alcohol during pregnancy. Because only 1.2% (2 women) of the sample were non–daily (occasional) smokers, and 100% reported never drinking during pregnancy, these variables were not examined in the analyses.

2.3. Statistical analyses

Results are expressed as mean and median values; variability is indicated by SD and/or value range. All continuous data are represented as means (SD, standard deviation), all categorical data as numbers and percent. Frequency rates were compared by $\chi^2$ test. The correlation among variables was performing by using the Pearson correlation coefficient or ordinal regression, when normality assumption was satisfied, and for non-normal variables the Spearman correlation coefficient was used. The hypothesis of the dispersion equality was verified using Levene’s test.

Study I. Following descriptive variables, skewness was estimated by the Kolmogorov-Smirnov test: $p<0.05$ was considered to indicate a non-Gaussian distribution. TSH showed a left skewed non-Gaussian and values
of FT4 concentrations a near normal distribution. The most powerful transformation to reduce the positive skew was square root transformation. TSH was normalized using square root transformation in order to use parametric tests and summarized as means with 95% confidence intervals (CI). Transformed mean values were untransformed for presentation. Means were compared with t-tests and analysis of variance.

General linear models (GLM) using both the repeated measures ANOVA (three time points) and the mixed model for the repeated measures ANOVA (three time points, two groups - TPO-Ab negative and positive), were used to explore the longitudinal effect of pregnancy (followed by Bonferroni post hoc pair-wise comparisons) and the effect of elevated TPO-Ab on the thyroid axis hormone concentrations (F test was reported). The comparisons in TPO-Ab positive and negative mothers among trimesters were made by GLM univariate ANOVA followed by pair-wise comparisons. For two group comparisons, ANOVA will give results identical to a t-test. The F tests the effect of TPO-Ab. Effect size for the repeated measures ANOVA was measured by eta squared $\eta^2$. A p-value of $<0.05$ was considered significant.

The reference intervals of the hormone concentrations are reported as median and empirical (based on the order statistics) 2.5th–97.5th percentiles by trimester. The International Federation of Clinical Chemistry (IFCC) recommends at least 120 reference values to use for the calculation of a reference interval (denoted the 0.95 central inter-fractile interval and defined as the interval between the 0.025 and the 0.975 fractiles of the distribution). For most purposes, non-parametric intervals are recommended (304). The trimester-specific TSH reference intervals were calculated with TPO-Ab positive women excluded and included.

Study II. Mean, median and standard deviation (SD) of TSH values of the newborns were considered to report the result of the screening. The Mann–Whitney test was used to detect difference in median by groups defined by newborn gender. Newborns TSH complied with assumptions of normality of the dependent variable. The effects of maternal age, gestational age, gender and birth weight on newborn TSH levels were assessed by analysis of variance (ANOVA), multiple correlation coefficient, the Spearman’s correlation coefficients. Since birth weight is dependent on gestational age, we conducted a multivariate analysis simultaneously to examine the relationship between neonatal TSH levels and maternal age (modeled continuously), newborn gender, gestational age and birth weight. Multiple linear regressions were performed to quantify the associations between the above parameters and change in TSH.
Study III. The linear regression analysis was used to examine whether maternal age and pre–pregnancy weight, gestational age, thyroid function in all three trimesters of pregnancy predicted anthropometric characteristics and Apgar scores of newborns. Five separate regression models were created for neonatal weight, height, BMI and Apgar scores in the 1st and 5th minutes of extrauterine life. Linear and quadratic terms (a quadratic term for maternal age was included in the final analysis to improve model fit) of maternal age at the time of delivering the birth, gestational age, pre–pregnancy weight, number of gestations, duration of pregnancy, gender of the newborn, TSH and FT4 values in all three trimesters of pregnancy were used as independent variables. Parity, the number of times a woman had previously given birth, was dichotomized into 0 – nulliparous or 1 – multiparous. This set of variables was reduced by backward elimination until only those significant at p < 0.05 remained in the model. Using the determination coefficient R² value and the model correspondence criterion, we created the optimal regression model allowing for prognosis of anthropometrics of newborns. Factors that were not statistically significant and worsened the R² value were excluded from the final regression model.

Study IV, V. For comparison of score on the EDS, STAI, acute and chronic stress among three trimesters, the non parametric tests (Friedman test) were applied.

The linear regression analyses were used to examine whether maternal age and pre-pregnancy weight, gestational age, thyroid function, symptoms of depression, symptoms of anxiety, perceived acute and chronic psychosocial stress in all three trimesters of pregnancy and personality traits in the second trimester of pregnancy predicted anthropometric characteristics and Apgar scores of newborns. Five separate regression models were created for neonatal weight, height, BMI and Apgar scores in the 1st and 5th minutes of extra-uterine life. Linear and quadratic terms (a quadratic term for maternal age was included in the final analysis to improve model fit) of maternal age at the time of delivering the birth, gestational age, pre-pregnancy weight, number of gestations, duration of pregnancy, gender of the newborns, TSH and FT4 values, score on the EDS, scores on the STAI, perceived acute and chronic psychosocial stress in all three trimesters of pregnancy and score on the BFPI in the second trimester of pregnancy were used as independent variables. The dichotomous variables were created with values of acute and chronic stress; non-stress category included all participants who not identified themselves as stressed.

This set of variables was reduced by backward elimination until only those significant at p < 0.05 remained in the model. Using the determination coefficient R² value and the model correspondence criterion, we created the
optimal regression model allowing for prognosis of anthropometrics of newborns. Factors that were not statistically significant and worsened the $R^2$ value were excluded from the final regression model. A probability level of $p<0.05$ was considered as statistically significant. All statistical analyses were performed using software from Statistical Package for Social Sciences 15.0 for Windows (SPSS, Inc., Chicago, Ill., USA).
3. RESULTS

3.1. Maternal thyroid function tests during pregnancy (Study I)

3.1.1. Reference intervals for thyroid testing at each trimester of pregnancy

The descriptive statistical measures of serum TSH and FT4 concentrations in terms of mean, median and standard deviation, 2.5th and 97.5th percentiles in healthy pregnant women negative for TPO–Ab in three trimesters of pregnancy are presented in Table 3.1.1.1. The reference intervals for nonpregnant adults provided by the manufacturer are also included for comparison.

*The reference intervals for TSH concentrations* were found to be 0.02–2.72 mIU/L for the first trimester, 0.22–2.51 mIU/L for the second trimester and 0.28–2.36 mIU/L for the third trimester of pregnancy.

*The reference intervals for FT4 concentrations* were determined to be 13.2–23.1 pmol/L for the first trimester, 11.8–18.5 pmol/L for the second trimester and 10.5–18.3 pmol/L for the third trimester of pregnancy.

Table 3.1.1.1. Descriptive statistics of TSH and FT4 concentrations for each trimester of pregnancy in healthy women negative for TPO-Ab (N=161)

<table>
<thead>
<tr>
<th>TSH, mIU/L</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.83</td>
<td>1.04</td>
<td>1.00</td>
</tr>
<tr>
<td>Median</td>
<td>0.70</td>
<td>0.93</td>
<td>0.91</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.67</td>
<td>0.57</td>
<td>0.51</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.003</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.82</td>
<td>3.78</td>
<td>3.03</td>
</tr>
<tr>
<td>Skewness</td>
<td>2.29</td>
<td>0.49</td>
<td>1.44</td>
</tr>
<tr>
<td>Kolmogorov–Smirnov test</td>
<td>0.014</td>
<td>0.002</td>
<td>0.056</td>
</tr>
<tr>
<td>Kolmogorov–Smirnov test after square root transformation</td>
<td>0.823</td>
<td>0.079</td>
<td>0.412</td>
</tr>
<tr>
<td>Trimester specific reference intervals, calculated as 2.5 – 97.5 percentiles</td>
<td>0.02–2.72</td>
<td>0.22–2.51</td>
<td>0.28–2.36</td>
</tr>
<tr>
<td>Manufacturer’s nonpregnant adult reference interval</td>
<td>0.4–4.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3.1.1.1 (continued)

<table>
<thead>
<tr>
<th>FT4, pmol/L</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>17.15</td>
<td>14.57</td>
<td>13.97</td>
</tr>
<tr>
<td>Median</td>
<td>17.00</td>
<td>14.50</td>
<td>13.90</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.36</td>
<td>1.75</td>
<td>1.80</td>
</tr>
<tr>
<td>Minimum</td>
<td>12.20</td>
<td>11.20</td>
<td>8.64</td>
</tr>
<tr>
<td>Maximum</td>
<td>23.70</td>
<td>22.20</td>
<td>21.20</td>
</tr>
<tr>
<td>Skewness</td>
<td>0.80</td>
<td>1.13</td>
<td>0.37</td>
</tr>
<tr>
<td>Kolmogorov–Smirnov test</td>
<td>0.565</td>
<td>0.432</td>
<td>0.831</td>
</tr>
<tr>
<td>Trimester specific reference intervals, calculated as 2.5 – 97.5 percentiles</td>
<td>13.2–23.1</td>
<td>11.8–18.5</td>
<td>10.5–18.3</td>
</tr>
<tr>
<td>Manufacturer’s nonpregnant adult reference interval a</td>
<td>10.3–25.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The IMMULITE Third generation (Diagnostic Products Corporation, Los Angeles, USA) analyzer was used for testing. TSH, thyrotrophin; FT4, free thyroxine.

### 3.1.2. Trimester changes in concentration of TSH and FT4 during pregnancy

Serum TSH (based on square root–transformed value) and serum FT4 (arithmetic means) concentrations in 161 women without elevated TPO-Ab concentrations and in 23 pregnant women with elevated TPO-Ab concentration are presented in Figures 3.1.2.1 and 3.1.2.2.

A 3 (trimester) x 2 (TPO-Ab) mixed-model for the repeated measures ANOVA showed that there was a significant main effect on TSH for TPO-Ab, F(1,182)=15.19, p<0.001 (Figure 3.1.2.1). The interaction effect (trimester x TPO-Ab) is important and it is significant at p=0.002. Clearly, the TPO-Ab is having a differential effect on the two groups. Two analyses were run, one on each group. A repeated measures ANOVA determined that mean TSH concentration in the TPO-Ab negative women differed statistically between trimesters F(2,160)=25.34, p<0.001, though this was a relatively small effect size ($\eta^2=0.14$). Post hoc tests revealed that was a significant difference in TSH levels between trimesters 1 and 2, and 1 and 3 (p<0.001), but no significant difference between trimesters 2 and 3. The changes in TSH by trimester in TPO-Ab positive women were not significant (F(2,22)=1.35, p<0.27).
Figure 3.1.2.1. Thyroid stimulating hormone (TSH) concentrations by trimester of gestation in pregnant women with normal (n=161) and elevated (>35 IU/ml, n=23) TPO-Ab concentrations

Dots represent the normalized average of TSH in each trimester. A square root transformation was used (mean, 95% CI).

** p<0.01; * p<0.05 age adjusted differences between subjects positive and negative for TPO–Ab;

Comparison among trimesters:
TPO-Ab ≤ 35 IU/ml: I vs. II and III p<0.001; II vs. III p>0.05
TPO-Ab >35 IU/ml: I vs. II and III; II vs. III p>0.05

The results of comparisons among trimesters in TPO-Ab positive and negative mothers by GLM univariate ANOVA showed significant effect on TSH for TPO-Ab in the first and second trimesters, but not in the third trimester (p=0.071). After controlling for age (with age as covariate) the TPO-Ab positive women had significantly higher TSH concentrations compared to TPO-Ab negative in the first (F(1,183)=11.59, p=0.001); in the second (F(1,183)=12.45, p=0.001) and third trimester (F(1,183)=4.58, p=0.035).

The means (95% CI) of the square root transformed TSH concentrations for each trimester were 0.71 (0.62–0.81), 0.97 (0.88–1.06), and 0.94 (0.86–1.02) mIU/L, respectively (Figure 3.1.2.1).

Mixed-model for repeated data revealed that the main effect of TPO-Ab on FT4 was not significant F(1,182)=0.56, p=0.45 (Figure 3.1.2.2). Thus, there was no overall difference in the FT4 of TPO-Ab positive compared to TPO-Ab negative. The fact that the linear trend was not significant for the interaction (trimester x TPO-Ab) means that both groups are showing about the same linear trend. A significant main effect for trimester was obtained, F(1,182)=89.8, p<0.001, η²=0.33. Bonferroni comparisons revealed a significant difference in FT4 levels between trimesters 1 and 2, and 1 and 3, and 2 and 3 (p<0.001) in TPO-Ab negative women. In TPO-Ab positive
there was a significant difference in FT4 levels between trimesters 1 and 2, and 1 and 3 (p<0.05), but no significant difference between trimesters 2 and 3.

The means (95% CI) of FT4 concentrations for each trimester were 17.15 (16.78–17.52), 14.57 (14.30–14.84), and 13.97 (13.69–14.25) pmol/L, respectively. The physiologic decrement in the mean concentrations of FT4 reaching 19% was observed during the second and the third trimester of pregnancy compared to the first trimester of pregnancy in both groups of women (Figure 3.1.2.2).

Figure 3.1.2.2. Free T4 concentrations by trimesters of gestation in pregnant women with normal (n=161) and elevated (>35 IU/ml, n=23) TPO-Ab concentrations (mean, 95% CI)

The physiologic decrement of mean FT4 concentrations during pregnancy, 19%.
Comparison among trimesters:
TPO-Ab ≤ 35 IU/ml: I trimester vs. II and III; II vs. III p<0.001;
TPO-Ab >35 IU/ml: I trimester vs. II and III p<0.05; II vs. III p>0.05.

Gestational age-specific reference intervals in antibody negative women for each assay are shown in Table 3.1.1.1. These reference intervals were used to classify TFT results (e.g. ‘high’=above 97.5th confidence limit, ‘normal’=within central 95% confidence interval, and ‘low’=below 2.5th confidence limit), and then were compared with classifications determined using the non-pregnant assay-specific reference intervals provided by the assay manufacturer (Table 3.1.2.1).
Table 3.1.2.1. Serum TSH and FT4 concentrations in pregnancy outside the reference intervals (RI)

<table>
<thead>
<tr>
<th></th>
<th>Trimester of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
</tr>
<tr>
<td>Outside non-pregnant RI (0.4-4.5 mIU/L)</td>
<td></td>
</tr>
<tr>
<td>&lt;Lower limit, n (%)</td>
<td>40 (24.8)</td>
</tr>
<tr>
<td>&gt;Upper limit, n (%)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Outside of gestation-related RI</td>
<td></td>
</tr>
<tr>
<td>&lt;Lower limit, n (%)</td>
<td>3 (1.9)</td>
</tr>
<tr>
<td>&gt;Upper limit, n (%)</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>Misclassified &lt;Lower limit</td>
<td>37 (22.9)</td>
</tr>
<tr>
<td>Not identified &gt;Upper limit</td>
<td>4 (2.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Trimester of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd</td>
</tr>
<tr>
<td>Outside non-pregnant RI (10.3-25.7 pmol/L)</td>
<td></td>
</tr>
<tr>
<td>&lt;Lower limit, n (%)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;Upper limit, n (%)</td>
<td>0</td>
</tr>
<tr>
<td>Outside of gestation-related RI</td>
<td></td>
</tr>
<tr>
<td>&lt;Lower limit, n (%)</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>&gt;Upper limit, n (%)</td>
<td>3 (1.4)</td>
</tr>
<tr>
<td>Misclassified &lt;Lower limit</td>
<td>0</td>
</tr>
<tr>
<td>Not identified &gt;Upper limit</td>
<td>0</td>
</tr>
</tbody>
</table>

RI, non-pregnant reference interval: TSH, 0.4-4.5 mIU/L; FT4, 10.3-25.7 pmol/L

When the non–pregnant TSH reference interval (0.4–4.5 mIU/L) was applied to the study participants, 37 women (22.9%) whose serum TSH concentration was within the first–trimester–specific reference range would have been misclassified as having subclinical hyperthyroidism, and four women (2.5%) with a TSH concentration above the first–trimester–specific upper reference limit would not have been identified. In the second trimester four women (3.7%) would have been misclassified and three (1.4%) had gone unidentified while during the third trimester 10 (6.2%) would have been misclassified and three (1.4%) not been identified. One woman in the first trimester had a concentration of TSH over 4.5 mIU/L suggesting subclinical hypothyroidism; however, her FT4 concentrations were high nor-
mal. Moreover, her TSH and FT4 concentrations in the second and in the third trimesters were also in the normal range confirming euthyroidism.

Figure 3.1.2.3, Figure 3.1.2.4 and Figure 3.1.2.5 show the plotted serum TSH concentrations versus FT4 concentrations data against gestational age in pregnant women with normal (n=161) and with elevated (n=23) TPO-Ab antibody concentrations (>35 IU/mL) in three trimesters of pregnancy.

Figure 3.1.2.3. The spreadsheet showing distribution of FT4 versus TSH serum concentrations in pregnant women with normal (n=161) and elevated (>35 IU/ml, n=23) TPO-Ab concentrations in the 1st trimester of pregnancy. The solid lines show manufacturers’s reference interval for nonpregnant adults, the dotted lines show trimester specific distribution in healthy pregnant women.
Figure 3.1.2.4. The spreadsheet showing distribution of FT4 versus TSH serum concentrations in pregnant women with normal (> 35 IU/ml, n=161) and elevated (n=23) TPO-Ab concentrations in the 2nd trimester of pregnancy. The solid lines show manufacturers's reference interval for nonpregnant adults, the dotted lines show trimester specific distribution in healthy pregnant women.
Figure 3.1.2.5. The spreadsheet showing distribution of FT4 versus TSH serum concentrations in pregnant women with normal (n=161) and elevated (>35 IU/ml, n=23) TPO-Ab concentrations in the 3rd trimester of pregnancy. The solid lines show manufacturers’s reference interval for nonpregnant adults, the dotted lines show trimester specific distribution in healthy pregnant women.

The individual plotted values (filled circles) represent the results in the 23 pregnancy cases with positive TPO-Ab of whom there were two (8.6%) value in the first trimester, and 4 (17.3%) value in both second and third trimester clearly outside (to the right) of the 97.5 percentile range of serum TSH concentration for TPO-Ab negative pregnant women.

A linear relationship between the log transformed TSH and FT4 values during pregnancy was observed (Figure 3.1.2.6).
Figure 3.1.2.6. Scatter plots of TSH vs. FT4 (based on logarithmically-transformed value) at 12–16 (A), 22–26 (B) and 32–36 (C) week of gestation in pregnant women with normal (open circle, n=161) and elevated (filled circle, n=23) TPO-Ab concentrations (>35 IU/ml) in the three trimesters of pregnancy.
Correlations between the two hormone concentrations were *moderate in the first trimester* (A) at 12–16 wk \( (r^2=0.125, r=-0.356, p<0.001) \) and weak in the two others: (B) at 22–26 week \( (r^2=0.045, r=-0.21, p=0.004) \) and (C) at 32–36 week \( (r^2=0.018, r=-0.136, p=0.066) \) indicating that FT4 concentrations were poorly predicted by TSH concentrations during second and third trimesters of pregnancy (Figure 3.1.2.6).

### 3.1.3. Effect of thyroid peroxidase antibodies on thyroid stimulating hormone reference limits

TSH and FT4 reference ranges were calculated when TPO-Ab positive patients were included in reference population.

**Table 3.1.3.1.** TSH, FT4 – median, 2.5th and 97.5th percentiles in the pregnant women after including those positive for thyroid peroxidase (TPO-Ab) antibody \( (N=184) \)

<table>
<thead>
<tr>
<th>TSH, mIU/L</th>
<th>FT4, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimester of gestation</td>
<td>1st</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Median</td>
<td>0.76</td>
</tr>
<tr>
<td>2.5 percentile</td>
<td>0.02</td>
</tr>
<tr>
<td>97.5 percentile</td>
<td>2.91</td>
</tr>
</tbody>
</table>

TSH, thyroid stimulating hormone; FT4, free thyroxine

The lower TSH reference limit was not affected by the inclusion of TPO-Ab positive women, but resulted in higher upper reference limits during pregnancy. On including the 23 pregnancies cases with positive TPO-Ab, the 97.5 percentiles for TSH increased to 2.91, 2.82 and 2.52 mIU/L, respectively (Table 3.1.3.1).

In pregnant women without a history of thyroid disease the median, 2.5 and 97.5 percentiles for TSH (mIU/L) were 0.70 and 0.02–2.72 (first trimester), 0.93 and 0.22–2.51 (second trimester), 0.91 and 0.28–2.36 (third trimester). On including women with positive TPO-Ab, the 97.5 percentiles for TSH increased to 2.91, 2.82 and 2.52 mIU/L, respectively.

### 3.2. The newborn thyroid stimulating hormone (Study II, III)

The data obtained for newborn TSH showed Gaussian distribution, enabling the paired *t* test to be used to compare the mean measurements. Histo-
gram showing the Gaussian distribution of newborns TSH (K–S test p=0.1) is presented (Figure 3.2.1).

![Histogram showing the Gaussian distribution of newborns TSH, mIU/L (Kolmogorov–Smirnov test, p=0.1)](image)

**Figure 3.2.1.** Histogram showing the Gaussian distribution of newborns TSH, mIU/L (Kolmogorov–Smirnov test, p=0.1)

The study sample consisted of 173 newborns. The median, interquartile ranges, range of the TSH at the third day of life, gestational age and birth weight of the 96 male and 77 female newborns are presented in Table 3.2.1. There was 1 (1.1%) male newborn born at <37 weeks of gestation (maternal age 21 year, gestational age 35 week, birth weight 2176 g, TSH 2.21 mIU/L). Two newborns, one female and one male, were with birthweight less than 2500 g. The values of TSH were 2.21 (female, gestation age 39 week, birth weight 2176 g, Apgar 9 at 5 minutes) and 3.47 mIU/L (male, 36 week, 2176 g, Apgar 9 at 5 minutes). There was no gender effect of newborns on birthweight (F(1,172)=2.4; p=0.121).

**Table 3.2.1.** Descriptive continuous data showing the median, IQR\(^2\) and range (minimum and maximum values) for newborn TSH, birthweight and gestational age in male and female infants

<table>
<thead>
<tr>
<th></th>
<th>All (N=173)</th>
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<tbody>
<tr>
<td></td>
<td>mediana</td>
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<tr>
<td>TSH, mIU/L</td>
<td>3.23</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>3540</td>
</tr>
<tr>
<td>Gestation, weeks</td>
<td>40</td>
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</table>
Table 3.2.1. (continued)

<table>
<thead>
<tr>
<th></th>
<th>Males (M) N=96</th>
<th>Females (F) N=77</th>
<th>p&lt;sup&gt;b&lt;/sup&gt; M vs. F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH, mIU/L</td>
<td>mediana IQR (range)</td>
<td>mediana IQR (range)</td>
<td></td>
</tr>
<tr>
<td>3.38</td>
<td>3.26–3.95 (0.23–6.03)</td>
<td>3.12</td>
<td>2.28–3.59 (1.05–5.37)</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>3586 (2176–4650)</td>
<td>3500 (2176–4410)</td>
<td>0.075</td>
</tr>
<tr>
<td>Gestation, weeks</td>
<td>40 (39–40) (35–41)</td>
<td>40 (39–40) (37–41)</td>
<td>0.284</td>
</tr>
</tbody>
</table>

TSH, thyrotrophin

<sup>a</sup>IQR, interquartile range, the range from the 25th to 75th percentile

<sup>b</sup>Mann–Whitney tests

In study III, IV, V 125 women had vaginal delivery and 44 (25%) underwent cesarean section. Newborn delivery mode data are presented Table 3.2.2.

Mean (SD) infant weight was 3.55 (0.45) kg, mean newborn TSH was 3.1 mIU/L (SD 1.0). The median concentrations (and interquartile ranges) of TSH in babies born by Cesarean section (25%), was 3.19 (IQR 1.37) mIU/L. This value was not significantly different from the corresponding ones in babies born by normal vaginal delivery (p=0.73) (Table 3.2.2)

Table 3.2.2. Newborn TSH according to the mode of delivery

<table>
<thead>
<tr>
<th>Delivery type</th>
<th>Newborn TSH N=173</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>Normal vaginal</td>
<td>3.33</td>
</tr>
<tr>
<td>Vacuum extraction</td>
<td>2.37</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>3.19</td>
</tr>
<tr>
<td>Cesarean section, N=44</td>
<td></td>
</tr>
<tr>
<td>Emergency cesarean&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.53</td>
</tr>
<tr>
<td>Elective cesarean</td>
<td>2.8</td>
</tr>
<tr>
<td>Breech presentation</td>
<td>2.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>IQR, interquartile range, the range from the 25th to 75th percentile

<sup>b</sup>Mann-Whitney U test between acute and elective cesarean section p=0.056

TSH in babies delivered by emergency Cesarean section tended to be higher than in delivered by elective Cesarean section (median, 3.53 versus 2.8 mIU/L, p=0.056). The differences of TSH concentration in babies deli-
tered by normal, vacuum extraction or Cesarean section were not significant (Kruskal-Wallis test \( p=0.18 \)) (Table 3.2.2).

The mode of delivery does not have considerable influence on TSH in the newborns, although minor differences exist. The mean serum TSH level following elective cesarean section was lower (but not significantly) than after normal vaginal delivery.

All newborn TSH values were below cut-off value for congenital hypothyroidism (10 mIU/L). The prevalence of newborn thyrotropin >5 mIU/L (%) was 2.8 % (four males and one female). The values were from 5.03 to 6.03 mIU/L. Figure 3.2.2 shows cumulative frequencies of TSH values.

![Figure 3.2.2. Cumulative frequency of the TSH of the newborns](image)

The distribution of TSH was close to statistical significance different between males and females, with males tended to have a higher median TSH (3.38 compared with 3.12 in females, \( p=0.054 \)).

Median (range) first minute Apgar score was 9 (6–10) and fifth minute was 9.5 (8–10). Apgar scores at 1 minute were not related to neonatal TSH, however, there was Apgar scores at 5 minutes effect on TSH (gender adjusted \( F(2,162)=4.9; p=0.027 \)). Infants with Apgar of \( \leq 8 \) at 5 minutes had higher levels of TSH as compared to those with Apgar of 9–10 at 5 minutes (mean 3.78 ± 1.02 and 3.06 ± 1.04, respectively; \( p=0.027 \)).
3.2.1. Iodine deficiency assessed by the newborn TSH concentrations (Study II)

Whole-blood TSH values were available for 213 newborns. TSH value ranged 0.23–6.03 mIU/L, with a median of 3.23 (interquartile range, 1.17 mIU/L), mean (SD) - 3.14 (0.99). A total of 6 newborns (2.8%) had TSH values >5 mIU/L.

Age-specific 2SD range (mean ± 2SD) necessary for interpretation of newborn TSH levels at the third day of life was established 1.15–5.13 mIU/L.

3.2.2. The newborn TSH levels in relation to maternal age, gestation, birthweight and gender (Study III)

The descriptive newborn data, medians and interquartile ranges (IRQ) are presented in Table 3.2.1.

An effect of gestational age on TSH levels was established at the p-value below 0.1 (β=0.14, p=0.07). Gender specific analyses showed that gestational age was not correlated with TSH levels when stratified by gender (p=0.41 and 0.33 for males and females, respectively).

![Figure 3.2.2.1. Thyroid stimulating hormone (TSH) value in infants with different gestational age](image)

There were also no effects of birth weight on newborn TSH levels (β=0.02, p=0.871). Two newborns were lighter than 2500 grams at birth.
The values of TSH were 2.21 (female, gestation age 39 week, birthweight 2176 g) and 3.47 mIU/L (male, 36 week, 2176 g).

Newborn TSH levels were associated with maternal age ($\beta=0.17$, $p=0.029$). Spearman rank correlation analysis showed a significant positive correlation between birth weight and gestational age ($\rho=0.398$, $p<0.001$). Sex–specific analyses showed similar correlation coefficients as for the combined analysis ($p<0.001$ and $p=0.006$ for males and females, respectively). There was no gender effect of newborns on birthweight ($F(1,168)=2.4; p=0.121$) and on maternal age ($F(1,168)=1.1; p=0.287$). Gestational age correlated significantly with maternal age ($\rho=0.15$, $p=0.032$) and birth weight ($\rho=0.33$, $p<0.001$).

**Multivariate analyses.** Since birth weight is dependent on gestational age, we conducted a multivariate analysis *simultaneously* to examine the relationship between neonatal TSH levels and maternal age (modeled continuously), newborn gender, gestational age and birth weight. The outcome variable in the analysis was the TSH level and the results are shown in Table 3.2.2.1. The model ($F(3,170)=2.99; p=0.020$) showed a significant adjusted associations with TSH levels for maternal age ($p=0.022$) and gestational age ($p=0.035$), but not for birth weight ($p=0.267$), and newborn gender ($p=0.086$).

**Table 3.2.2.1. Adjusted coefficients for factors simultaneously included in a multiple linear regression model with the newborn TSH as the outcome variable**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Beta ($\beta$)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, year</td>
<td>0.18</td>
<td>0.022</td>
</tr>
<tr>
<td>Gestational age, week</td>
<td>0.18</td>
<td>0.035</td>
</tr>
<tr>
<td>Gender (1, female; 2, male)</td>
<td>0.13</td>
<td>0.086</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>−0.09</td>
<td>0.267</td>
</tr>
</tbody>
</table>

Dependent variable: newborn TSH; $\beta$ – standardized regression coefficient
R$^2$ adjusted = 0.069; $p<0.02$
Adjusted for maternal age, child gender, gestational age, and birth weight

Gestational age was associated with TSH level in multivariate but not in univariate models. Positive association between maternal age and newborn TSH levels persisted in both univariate and multivariate models. In this model for TSH levels, the inclusion of all predictive covariates explained 6.9% of the total variation.
Linear multivariate regression analysis was performed in order to test the relationships between TSH level, newborn birth weight, newborn gender, and gestational age yielded the following regression equation:

\[
\text{TSH (mIU/L) = } [-0.00022 \times \text{birth weight (g)}] + [0.182 \times \text{gestational age (weeks)}] + [0.038 \times \text{maternal age (years)}] + [0.279 \times \text{gender (1, female; 2, male)}] - 4.86 \quad (\text{R}^2 = 0.069).
\]

The effect of birth weight and newborn gender was not significant.

3.2.3. The newborn TSH levels in relation to maternal thyroid function

When the neonatal and maternal thyroid function was compared (173 pair mother–newborn) the median neonatal serum concentrations of TSH were significantly higher than the maternal values in all trimesters of pregnancy (all \(p<0.001\)) (Table 3.2.3.1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>((N = 173))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal FT4 first trimester (SD)</td>
<td>17.12 (2.27)</td>
</tr>
<tr>
<td>Mean maternal TSH first trimester (SD)</td>
<td>0.91 (0.7)</td>
</tr>
<tr>
<td>Prevalence of TPO–Ab &gt;35 U/mL, %</td>
<td>12.5</td>
</tr>
<tr>
<td>Mean maternal FT4 second trimester (SD)</td>
<td>14.55 (1.73)</td>
</tr>
<tr>
<td>Mean maternal TSH second trimester (SD)</td>
<td>1.12 (0.65)</td>
</tr>
<tr>
<td>Mean maternal FT4 third trimester (SD)</td>
<td>13.93 (1.78)</td>
</tr>
<tr>
<td>Mean maternal TSH third trimester (SD)</td>
<td>1.04 (0.56)</td>
</tr>
<tr>
<td>TSH of infant heel blood sample</td>
<td>3.13 (1.00)</td>
</tr>
</tbody>
</table>

Notes: Maternal serum TSH (based on square root–transformed value) and serum FT4 (arithmetic means) concentrations

Mean maternal FT4 at 2nd trimester was higher in women who delivered male than female newborns (14.6 and 14.2 pmol/L, respectively; \(p=0.011\)).

There were no significant correlations between TSH of infant heel blood samples and maternal TSH (both square root and log transformed) and FT4 in the first, second, or third trimester. Infant heel blood samples for TSH were significantly correlated with gestational age (\(r=0.2\); \(p=0.042\)) but not with TPO-Ab, gender, and birth weight.

Gestational age and birth weight were also highly intercorrelated in newborns. Therefore, only the former was included in multiple regression mod-
els, where appropriate, because it was a better predictor of TSH concentrations. The data were analysed by multiple regression using as regressors maternal age, gestational age, birth weight, sex, the maternal concentrations of TSH or FT4 during all three trimesters and TPO-Ab measured in early pregnancy (modeled categorical: negative and positive). The overall relationship was significant ($F(3,170)=2.79$, $p<0.05$) only for model with maternal and gestational age, and maternal TPO-Ab as regressors. The effect of maternal and gestational age on newborn TSH was significant ($t_{173}=2.02$, $p<0.05$ and $t_{173}=2.18$, $p<0.05$, respectively). Controlling for maternal TSH and FT4 the models were not statistically significant.

Table 3.2.3.2. Adjusted coefficients for factors included in a multiple linear regression model with the newborn TSH as the outcome variable

<table>
<thead>
<tr>
<th>Model</th>
<th>Factor</th>
<th>Beta</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>–1.034</td>
<td>0.302</td>
<td>0.702</td>
</tr>
<tr>
<td></td>
<td>Maternal age, years</td>
<td>0.149</td>
<td>1.984</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>Gestational age, wk</td>
<td>0.153</td>
<td>2.038</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>R² = 0.032</td>
<td>F = 3.858</td>
<td>p = 0.023</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(Constant)</td>
<td>–1.200</td>
<td>0.232</td>
<td>0.702</td>
</tr>
<tr>
<td></td>
<td>Maternal age, years</td>
<td>0.152</td>
<td>2.018</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Gestational age, wk</td>
<td>0.170</td>
<td>2.182</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>TPO–Ab, negative</td>
<td>–0.065</td>
<td>–0.834</td>
<td>0.406</td>
</tr>
<tr>
<td></td>
<td>R² = 0.049</td>
<td>F = 2.79</td>
<td>p = 0.038</td>
<td></td>
</tr>
</tbody>
</table>

In this study of 173 children born with normal thyroid function, we saw no evidence that newborn TSH concentrations were associated with maternal TSH, FT4, or elevated TPO-Ab. The effect of maternal and gestational age on newborn TSH was significant.
3.3. Maternal thyroid function during pregnancy in the interference with anthropometric parameters of newborn

A total of 173 birth delivery medical case histories were analyzed. Correlation analysis (Table 3.3.1 by variable) revealed that gestational age and all maternal anthropometric measurements (and BMI) were positively correlated (p<0.01) with birth weight and height. Older *maternal age* was related to greater maternal BMI (but not to weight and height separately).

*Table 3.3.1. Correlations between the primary variables*

<table>
<thead>
<tr>
<th></th>
<th>Newborn weight</th>
<th>Newborn height</th>
<th>Apgar 1 min.</th>
<th>Apgar 5 min.</th>
<th>Maternal age</th>
<th>Gestational age</th>
<th>Parity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>1</td>
<td>0.57**</td>
<td>0.06</td>
<td>0.02</td>
<td>0.12</td>
<td>0.35**</td>
<td>–0.08</td>
</tr>
<tr>
<td>Height, cm</td>
<td>0.57**</td>
<td>1</td>
<td>–0.002</td>
<td>0.06</td>
<td>0.08</td>
<td>0.35**</td>
<td>–0.07</td>
</tr>
<tr>
<td>Apgar 1</td>
<td>0.05</td>
<td>–0.002</td>
<td>1</td>
<td>0.68**</td>
<td>0.11</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Apgar 5</td>
<td>0.02</td>
<td>0.06</td>
<td>0.68**</td>
<td>1</td>
<td>0.16*</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal age, years</td>
<td>0.12</td>
<td>0.08</td>
<td>0.11</td>
<td>0.16*</td>
<td>1</td>
<td>–0.10</td>
<td>0.52**</td>
</tr>
<tr>
<td>Gestational age, years</td>
<td>0.28**</td>
<td>0.31**</td>
<td>0.04</td>
<td>0.05</td>
<td>–0.10</td>
<td>1</td>
<td>–0.07</td>
</tr>
<tr>
<td>Gender (1, female; 2, male)</td>
<td>0.11</td>
<td>0.11</td>
<td>–0.06</td>
<td>0.004</td>
<td>0.07</td>
<td>–0.04</td>
<td>–0.04</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>0.21**</td>
<td>0.17*</td>
<td>–0.05</td>
<td>–0.08</td>
<td>0.162*</td>
<td>0.01</td>
<td>–0.03</td>
</tr>
<tr>
<td>Maternal height, cm</td>
<td>0.21**</td>
<td>0.14*</td>
<td>0.06</td>
<td>–0.04</td>
<td>–0.08</td>
<td>0.08</td>
<td>–0.08</td>
</tr>
<tr>
<td>Pre-pregnancy weight, kg</td>
<td>0.29**</td>
<td>0.22**</td>
<td>–0.02</td>
<td>–0.09</td>
<td>0.11</td>
<td>0.04</td>
<td>–0.03</td>
</tr>
<tr>
<td>Weight gain, kg/week</td>
<td>0.26**</td>
<td>0.11</td>
<td>0.02</td>
<td>–0.06</td>
<td>–0.13</td>
<td>0.01</td>
<td>–0.13</td>
</tr>
</tbody>
</table>

* parity, 0 – nullipara, 1 – multipara
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

The results of univariate linear regression analysis of the associations of birthweight with maternal TSH and FT4 levels by trimesters (n=173) are shown in Table 3.3.2.
Table 3.3.2. Univariate analysis of the relationship of birthweight with maternal thyroid function parameters by trimesters (n = 173)

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Maternal TSH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Maternal FT4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>Sig.</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>-0.09</td>
<td>0.23</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>0.02</td>
<td>0.82</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>-0.05</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Dependent variable: newborn birthweight; β – standardized regression coefficient
<sup>a</sup>after square root transformation

Of maternal thyroid function parameters only FT4 concentration at 2nd trimester was negatively correlated with newborn birthweight (β=−0.16, p=0.036).

Figure 3.3.1. Relationship between maternal age and newborn birthweight (filled circle, male gender)
Figure 3.3.2. Relationship between maternal age (above), gestational age (bottom) and birthweight (filled circle, male gender)

Figure 3.3.3. Relationship between maternal pre–pregnancy weight and birthweight

In the multivariate models for predicting newborn antropometric and maternal thyroid function parameters (TSH and FT4) in all trimesters of pregnancy, linear and quadratic terms (a quadratic term for gestational age and for maternal age was included in the final analysis to improve model fit) of maternal age at the time of delivering the birth (Figure 3.3.1–3.3.3), number of gestations, duration of pregnancy, gender and TSH of the newborn were used as independent variables.
Table 3.3.3. Multiple linear regression analysis of newborn birth weight, height, BMI and Apgar

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta&lt;sup&gt;a&lt;/sup&gt;</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth weight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.265, adjusted R² = 0.239</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (6, 170) = 10.76 p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre–pregnancy weight, kg</td>
<td>0.240</td>
<td>3.606</td>
<td>0.000</td>
</tr>
<tr>
<td>FT₄ concentration at 2nd trimester, pmol/L</td>
<td>–0.134</td>
<td>–2.014</td>
<td>0.046</td>
</tr>
<tr>
<td>Maternal age, year</td>
<td>–1.338</td>
<td>–2.055</td>
<td>0.041</td>
</tr>
<tr>
<td>Gestational age, week</td>
<td>7.404</td>
<td>2.543</td>
<td>0.012</td>
</tr>
<tr>
<td>Quadratic term of maternal age</td>
<td>1.413</td>
<td>2.170</td>
<td>0.031</td>
</tr>
<tr>
<td>Quadratic term of gestational age</td>
<td>–7.068</td>
<td>–2.427</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Birth height</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.194, adjusted R² = 0.176</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (4, 172) = 10.38 p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age, year</td>
<td>–1.519</td>
<td>–2.258</td>
<td>0.025</td>
</tr>
<tr>
<td>Quadratic term of maternal age</td>
<td>1.679</td>
<td>2.494</td>
<td>0.014</td>
</tr>
<tr>
<td>Gestational age, week</td>
<td>0.363</td>
<td>5.289</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight gain, kg</td>
<td>0.146</td>
<td>2.107</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Newborn’s BMI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.119, adjusted R² = 0.099</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (4, 172) = 5.81 p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT₄ concentration at 2nd trimester, pmol/L</td>
<td>–0.149</td>
<td>–2.071</td>
<td>0.040</td>
</tr>
<tr>
<td>Gestational age, week</td>
<td>6.447</td>
<td>2.076</td>
<td>0.039</td>
</tr>
<tr>
<td>Pre–pregnancy weight, kg</td>
<td>0.208</td>
<td>2.898</td>
<td>0.004</td>
</tr>
<tr>
<td>Quadratic term of gestational age</td>
<td>–6.291</td>
<td>–2.026</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>Apgar 1 score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.067, adjusted R² = 0.046</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (4, 172) = 3.1 p = 0.017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH concentration at 1st trimestre, mIU/L</td>
<td>0.156</td>
<td>2.101</td>
<td>0.037</td>
</tr>
<tr>
<td>FT₄ concentration at 3rd trimester, pmol/L</td>
<td>0.137</td>
<td>1.822</td>
<td>0.070</td>
</tr>
<tr>
<td>Gestational age, year</td>
<td>8.224</td>
<td>2.564</td>
<td>0.011</td>
</tr>
<tr>
<td>Quadratic term of gestational age, week</td>
<td>–8.253</td>
<td>–2.572</td>
<td>0.011</td>
</tr>
<tr>
<td><strong>Apgar 5 score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.143, adjusted R² = 0.122</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (1, 175) = 10.07 P &lt; 0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age, year</td>
<td>0.230</td>
<td>3.08</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<sup>a</sup> standardized coefficient; BMI, body mass index

68
Multivariate regression with backward elimination (Table 3.3.3.) revealed that gestational age ($\beta=7.4$, $p=0.012$), pre–pregnancy weight ($\beta=0.24$, $p<0.001$) were the strongest positive predictors of birth weight and FT4 concentrations in the 2nd trimester of pregnancy ($\beta=-0.134$, $p=0.046$) was negative predictor and together with maternal age and quadratic term for gestational age covered 23.9% of variance.

Weight gain of mother during pregnancy was a better predictor of newborn height than pre-pregnancy weight ($\beta=0.146$, $p=0.037$). Gestational age ($\beta=0.363$, $p<0.001$) was the strongest positive predictor of height of newborn whereas FT4 concentrations in the 2nd trimester of pregnancy ($\beta=-0.149$, $p=0.040$) was negative predictor of neonatal BMI and together with gestational age and pre–pregnancy weight covered 10% of variance.

Regardless of the Apgar scores in 5th postpartum minutes of extraterine life the protective effect of maternal age was observed as significant ($\beta=0.23$, $p=0.002$) and covered 12% of variance.

One of the thyroid function measurements, FT4 (but not TSH) concentration at 2nd trimester of pregnancy, was negatively affecting birth weight and BMI. TSH concentration at 1st trimester was positively associated with Apgar scores at first minute.

### 3.4. Antenatal maternal personality and mental state and anthropometric characteristics of the newborns (Study IV, V)

**Symptoms of depression** (EDS $\geq 12$) were found in 17 (9.8%) women in the first trimester of pregnancy, in 8 (4.6%) women in the second trimester of pregnancy and in 12 (6.9%) women in the third trimester of pregnancy. The differences in the prevalence of depressive disorder in the three phases of pregnancy were not statistically significant ($\chi^2=5.08$, df=2, $p=0.079$).

Of the all 173 women 146 (84.4%) did not perceive symptoms of depression during pregnancy, 20 (11.6%) were established as having symptoms of depression at least one trimester, 4 (2.3%) – at two trimesters, and 3 (1.7%) – at all three trimesters (Figure 3.4.1).

**Symptoms of anxiety** (45 and a more score in STAI) were found in 32 (18.5%), in 23 (13.3%) and in 17 (9.8%) pregnant women in the first, second and third trimesters of pregnancy, respectively. The differences in the decreasing prevalence of symptoms of anxiety in the three phases of pregnancy were statistically significant ($\chi^2=8.77$, df=2, $p=0.012$).

Of the all 173 women 128 (74%) did not perceived symptoms of anxiety during pregnancy, 24 (13.9%) were established as having symptoms of
anxiety at least one trimester, 15 (8.7%) – at two trimesters, and 6 (3.5%) – at all three trimesters (Figure 3.4.1).

Figure 3.4.1. Frequency of perceived symptoms of anxiety and depression during pregnancy

The differences in severity of acute stress among the three trimesters of pregnancy were not statistically significant (Friedman test, p=0.08). Severity of chronic stress was higher at first trimester, then decreased during second and then remained stable in the third (Friedman test, p<0.001).

Figure 3.4.2. Frequency of perceived acute and chronic stress during pregnancy

Of the all 173 women 35 (20.2%) at first trimester, 28 (16.5%) at second, and 22 (12.7%) at third were established as having acute stress, and 47 (27.2%), 30 (17.3%), 24 (13.9%) as having chronic stress, respectively (Figure 3.4.2).
Personality dimensions (Figure 3.4.3) were not significantly associated with both perceived acute or chronic stress. Conscientiousness was negatively related to depression and anxiety symptomology during pregnancy. Neuroticism was strongly related to anxiety and moderately to depression; extraversion was inversely associated with anxiety during pregnancy (Table 3.4.1).

Table 3.4.1. Big-Five personality dimensions relation with perceived symptomology of depression (by EDS) and anxiety (by STAI) by trimester of pregnancy

<table>
<thead>
<tr>
<th></th>
<th>EDS</th>
<th>STAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st trimester</td>
<td>2nd trimester</td>
</tr>
<tr>
<td>Extraversion</td>
<td>-0.09</td>
<td>-0.09</td>
</tr>
<tr>
<td>Agreeableness</td>
<td>-0.16*</td>
<td>-0.16*</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>-0.18*</td>
<td>-0.18*</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>0.40**</td>
<td>0.46**</td>
</tr>
<tr>
<td>Openness</td>
<td>-0.03</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01

The chronic stress in the second trimester correlated with newborn birthweight ($r=0.17$, $p=0.025$) and perceived acute stress in the third trimester correlated with Apgar scores in 5th minutes ($r=−0.17$, $p=0.029$).
We evaluated five maternal personality traits, but only one, namely conscientiousness, was positively associated with newborn birthweight and height (r=0.15 and r=0.22, respectively).

**Multivariate analysis**

Five separate regression models were created for neonatal weight, height, BMI and Apgar scores in the 1st and 5th minutes of extrauterine life using as regressors maternal age and pre-pregnancy weight, parity, gestational age, maternal TSH and FT4 concentration, perceived symptoms of depression and symptoms of anxiety, perceived acute and chronic psychosocial stress in all three trimesters of pregnancy and personality traits in the second trimester of pregnancy (Table 3.4.2).

Gestational age ($\beta=0.304$, $p=0.00$) and chronic stress in the 2nd trimester of pregnancy ($\beta=0.276$, $p=0.001$) were the strongest positive predictors whereas chronic stress ($\beta=-0.191$, $p=0.019$) and FT4 concentration ($\beta=-0.145$, $p=0.028$) in the 3rd trimester of pregnancy were the strongest negative predictors of birth weight and together with other significant variables covered 31% of variance (Table 3.4.2). Conscientiousness score in the 2nd trimester and EDS score in the 1st trimester of pregnancy were weak positive predictors of birth weight ($\beta=0.154$, $p=0.019$ and $\beta=0.143$, $p=0.033$; respectively).

Gestational age ($\beta=0.338$, $p=0.000$) and Conscientiousness score ($\beta=0.201$, $p=0.005$) were the strongest positive predictors of birth height and together with other significant variables covered 19% of variance.

Pre-pregnancy weight of mother ($\beta=0.210$, $p=0.004$), chronic stress in the 2nd trimester of pregnancy ($\beta=0.207$, $p=0.018$) and EDS score in the 1st trimester of pregnancy ($\beta=0.174$, $p=0.015$) were the strongest positive predictors of BMI of newborns whereas chronic stress in the 3rd trimester of pregnancy ($\beta=-0.198$, $p=0.023$) and FT4 concentrations in the 3rd trimester of pregnancy ($\beta=-0.165$, $p=0.023$) were negative predictors of neonatal BMI and together with gestational age covered 15% of variance.

Anxiety score in the 3rd trimester of pregnancy ($\beta=0.305$, $p=0.001$), maternal age ($\beta=0.268$, $p=0.000$) and neuroticism score ($\beta=0.267$, $p=0.004$) were positive predictors whereas acute stress in the 3rd trimester of pregnancy ($\beta=-0.177$, $p=0.016$) was negative predictor of Apgar score in the 5th minute of extrauterine life and covered 14% of variance.
**Table 3.4.2. Multiple linear regression analysis of newborn birth weight, height, BMI, Apgar scores**

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta, β</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth weight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.349, adjusted R² = 0.309</td>
<td></td>
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</tr>
<tr>
<td>F (10,161) = 8.630 p&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>Maternal age</td>
<td>-1.729</td>
<td>-2.656</td>
<td>0.009</td>
</tr>
<tr>
<td>Quadratic term of maternal age</td>
<td>1.806</td>
<td>2.779</td>
<td>0.006</td>
</tr>
<tr>
<td>Gestational age</td>
<td>0.304</td>
<td>4.680</td>
<td>0.000</td>
</tr>
<tr>
<td>Chronic stress at 2nd trimester</td>
<td>0.276</td>
<td>3.458</td>
<td>0.001</td>
</tr>
<tr>
<td>Pre-pregnancy weight</td>
<td>0.221</td>
<td>3.368</td>
<td>0.001</td>
</tr>
<tr>
<td><em>Chronic stress at 3rd trimester</em></td>
<td><strong>-0.191</strong></td>
<td><strong>-2.360</strong></td>
<td>0.019</td>
</tr>
<tr>
<td>Big Five Conscientiousness score</td>
<td>0.162</td>
<td>2.463</td>
<td>0.015</td>
</tr>
<tr>
<td>EDS score at 1st trimester</td>
<td>0.154</td>
<td>2.377</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>FT4 concentration at 3rd trimester</strong></td>
<td><strong>-0.145</strong></td>
<td><strong>-2.213</strong></td>
<td>0.028</td>
</tr>
<tr>
<td>Acute stress at 2nd trimester</td>
<td>0.143</td>
<td>2.153</td>
<td>0.033</td>
</tr>
<tr>
<td><strong>Birth height</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.211, adjusted R² = 0.192</td>
<td></td>
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<tr>
<td>F (4,167) = 11.182 p&lt;0.001</td>
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<tr>
<td>Maternal age</td>
<td>-1.843</td>
<td>-2.648</td>
<td>0.009</td>
</tr>
<tr>
<td>Quadratic term of maternal age</td>
<td>1.965</td>
<td>2.827</td>
<td>0.005</td>
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<tr>
<td>Gestational age</td>
<td>0.338</td>
<td>4.883</td>
<td>0.000</td>
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<tr>
<td>Big Five Conscientiousness score</td>
<td>0.201</td>
<td>2.879</td>
<td>0.005</td>
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<tr>
<td><strong>Newborn’s BMI</strong></td>
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<tr>
<td>R² = 0.175, adjusted R² = 0.145</td>
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<td></td>
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<tr>
<td>F (6,165) = 5.822 p&lt;0.001</td>
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<tr>
<td>Pre-pregnancy weight, kg</td>
<td>0.210</td>
<td>2.917</td>
<td>0.004</td>
</tr>
<tr>
<td>Chronic stress at 2nd trimester</td>
<td>0.207</td>
<td>2.382</td>
<td>0.018</td>
</tr>
<tr>
<td><em>Chronic stress at 3rd trimester</em></td>
<td><strong>-0.198</strong></td>
<td><strong>-2.292</strong></td>
<td>0.023</td>
</tr>
<tr>
<td>EDS score at 1st trimester</td>
<td>0.174</td>
<td>2.458</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>FT4 concentration at 3rd trimester</strong></td>
<td><strong>-0.165</strong></td>
<td><strong>-2.289</strong></td>
<td>0.023</td>
</tr>
<tr>
<td>Gestational age</td>
<td>0.159</td>
<td>2.216</td>
<td>0.028</td>
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<tr>
<td><strong>Apgar 5 score</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>R² = 0.143, adjusted R² = 0.122</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (4,167) = 6.949 P &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety score at 3rd trimester</td>
<td>0.305</td>
<td>-3.426</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.268</td>
<td>3.651</td>
<td>0.000</td>
</tr>
<tr>
<td>Big Five Neuroticism score</td>
<td>0.267</td>
<td>2.940</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Acute stress at 3rd trimester</em></td>
<td><strong>-0.177</strong></td>
<td><strong>-2.436</strong></td>
<td>0.016</td>
</tr>
</tbody>
</table>
4. DISCUSSION

4.1. Longitudinaly assessed maternal thyroid axis changes during pregnancy and comparisons of reference intervals

This longitudinal cohort study examining the pattern of serum TSH concentrations and FT4 concentrations in three trimesters of pregnancy in healthy, TPO-Ab negative women confirmed that thyroid axis hormone reference interval differs from assay specific referral ranges used in non-pregnant populations, supporting recommendations that trimester-specific reference ranges should be used (77). Moreover, changes in thyroid status in healthy Lithuanian women during pregnancy can be compared to similar studies for an in-land geographical area. The second and third trimesters upper reference limit found in our study corresponds to the TSH ranges proposed by the Endocrine Society Pregnancy Guidelines (8) and falls below 3.0 mIU/L. Though, our upper reference limit 2.72 mIU/L for the first trimester is higher than 2.5 mIU/L proposed by the Endocrine Society Pregnancy Guidelines.

Misdiagnosis of thyroid dysfunction during pregnancy may be crucial for mother and for child (248) (19). There is a controversy over the most reliable reference interval and cutoff values to differentiate normal thyroid function from abnormality associated with thyroid dysfunction (127). In clinical practice, several parameters have been identified to represent useful markers of thyroid function during pregnancy, including changes in serum TSH and in serum FT4 concentrations (305). During early pregnancy TSH secretion is suppressed by hCG induced by thyroid gland stimulation (79). During the second half of gestation, serum TSH concentrations return progressively to pre-pregnancy values and remain stable, unless additional underlying factors cause thyroid dysfunction. For instance, iodine deficiency or autoimmune thyroid disease may cause thyroid insufficiency resulting in progressive increase in serum TSH concentrations (306). Specifically, both the Belgian (78) and Danish (307) studies carried out in well defined areas with a marginal iodine deficiency, have showed an increase in serum TSH near term. The same investigators proved that iodine supplementation during pregnancy significantly prevented these alterations in serum TSH. Conversely, a Dutch study performed in an iodine replete area failed to show a discrepancy in serum TSH between the third trimester and nonpregnant values (308).

Using the assay specific reference interval for serum TSH one might suspect hyperthyroidism in normal women who have a low serum TSH value early in pregnancy, and one might miss hypothyroidism in women who have
a slight TSH elevation (205). With the assay specific reference interval for TSH almost a quarter of pregnant women might be incorrectly diagnosed with hyperthyroidism in the first trimester of pregnancy. In the second and third trimesters some women would also have been classified wrongly. Similar findings have been noted by Dashe et al. (139).

A direct comparison of our reference intervals to other published data is problematic for several reasons. Selection of normal subjects may account for variations in reference interval by different authors, despite the use of the same methodology for testing (137). The establishment of reference interval can be influenced significantly by age, ethnicity, season, cigarette smoking, exercise, sleep deprivation, fasting, phlebotomy timing, methods of analysis, iodine status, selection of subjects and calculation method (99, 120, 137, 309-313).

There is evidence that serum TSH concentration decreases markedly during the morning from 8:00 to 9:30 AM, thereafter the concentration remains relatively constant until evening, with a smaller nadir in the late afternoon (314). In this respect we performed blood sampling, trying to eliminate the effect of diurnal TSH variation. As time of sampling is unknown in most studies (315), sampling time differences between studies may be an important reason for the differences in published reference intervals. We obtained reference interval for FT4 and TSH from healthy and ethnically homogeneous pregnant women, as reference values tend to vary according to the ethnic group (99-100, 316). TSH concentrations decrease significantly in twin pregnancies compared to singleton during the first trimester (139). Therefore, only singleton pregnancies were included in our assessment. As FT3 was not measured, the full range of thyroidal metabolism during pregnancy could not be evaluated and hormonal measurement even earlier in the first trimester would have been of interest. Although our hormonal change observations were limited to the late first assessment (12–16 weeks), TSH reached a nadir in the first trimester, while later on, in the pregnancy, values increased, as been noted by other researchers (316). TSH suppression is associated with the elevation in circulating hCG (78-79, 317-318), peaking at around 11-12th gestational week, whereas estrogen production increases the mean TBG concentration 2–3 times the pre-pregnancy level by 16 to 20 weeks of gestation (23, 319), causing a shift in total T4 to approximately 1.5 times the non-pregnant level by 16 weeks (3, 23, 78). The latter, could partially explain our relatively high upper FT4 reference interval at 12–16 weeks or it might be the effect of iodine containing vitamine use during pregnancy, though we cannot provide the data on extent of their usage. Our lower FT4 reference interval was comparable to the study in Spain at 11–20 weeks (13.2 vs 13.1 pmol/L, respectively), but our upper limit was still
higher (23.1 vs 17.24 pmol/L, respectively) (320). First, trimester median FT4 values were 16.9 and 19.9 pmol/L, respectively, for the non-supplemented and potassium iodide supplemented women from the area of mild iodine deficiency in Spain (321).

Variations in reference interval for TSH and FT4 might be observed due to inclusion of different gestational weeks in trimesters and calculation methods used, also different manufacturer methods used.

There was a dearth of comparable data available in the literature because of many different methods used to calculate reference intervals. Reference intervals for TSH and FT4 varied according to different manufacturer methods and between authors, even when the same manufacturer method was applied. Table 4.1.1 shows the comparisons of reference intervals for TSH and FT4 between different authors and different manufacturer methods used for analysis. All reference intervals are trimester-based, and the same calculation method (i.e. a central 95% interval with the 2.5th and 97.5th percentile values) was used with the exception of three authors who used 5th and 98th percentile values, and 5th and 95th percentiles, respectively.

Compared with other authors, our data revealed a relatively lower cut–off values for the upper TSH reference limit, in particular when compared with the data reported by the authors that used the Immulite platforms. Some variations were also found in FT4 reference intervals. Our FT4 reference intervals had higher cut-off values (11.8–18.5 pmol/L) compared with the data reported by Lambert-Messerlian et al. (9.3–16.2 pmol/L), particularly during the second trimester.

Our reported reference interval, especially lower TSH and higher FT4 values in the first trimester is close to the ones observed from a previously mildly iodine-deficient area in Italy by Moleti et al. (322), in Spain by Boccos-Terraz et al. (320) and in China by Panesar et al. (81), compared to other authors (26, 38, 135, 323). The biggest disparity in the upper values of TSH reference intervals was observed in the above mentioned studies. Moreover, there are differences in reference intervals between authors, even when the same manufacturer method is applied, described by Yan et al. (137). We used Immulite analyzer and revealed lower TSH and higher FT4 reference intervals than described by two studies (136, 323) using the same analyzer.
Table 4.1.1. Comparisons of trimester–specific reference intervals for TSH and FT4 (data were calculated as 2.5th and 97.5th percentiles)

<table>
<thead>
<tr>
<th>Method used, authors</th>
<th>Analyte</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immulite 2000 analyzer</td>
<td>Manufacturer’s reference interval for nonpregnant adults: TSH: 0.4–4.0 mIU/L; 10.3–24.5 pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haddow et al. (USA, 2004)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>TSH</td>
<td>0.08–3.61</td>
<td>0.39–3.71</td>
<td></td>
</tr>
<tr>
<td>Lambert–Messerlian et al. (USA, 2008)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>TSH</td>
<td>0.12–3.37</td>
<td>0.35–3.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>10.4–17.8</td>
<td>9.3–16.2</td>
<td></td>
</tr>
<tr>
<td>Abbott ARCHITECT i2000 analyzer</td>
<td>Manufacturer’s reference interval for nonpregnant adults: TSH: 0.35–4.94 mIU/L; 9.0–19.1 pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stricker et al. (Switzerland, 2007)</td>
<td>TSH</td>
<td>0.09–2.83</td>
<td>0.20–2.79</td>
<td>0.31–2.90</td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>10.5–18.3</td>
<td>9.5–15.7</td>
<td>8.6–13.6</td>
</tr>
<tr>
<td>Boccos et al. (Spain, 2009)</td>
<td>TSH</td>
<td>0.03–2.57</td>
<td>0.12–2.64</td>
<td>0.23–3.56</td>
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<tr>
<td>Gilbert et al. (Australia, 2009)</td>
<td>TSH</td>
<td>0.02–2.15</td>
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<tr>
<td></td>
<td>FT4</td>
<td>10.4–17.8</td>
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<tr>
<td>Mannisto et al. (Finland, 2011)</td>
<td>TSH</td>
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<td>0.35–3.32</td>
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</tr>
<tr>
<td></td>
<td>FT4</td>
<td>11.86–21.85</td>
<td>11.2–18.86</td>
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<tr>
<td>Roche Elecsys 1010/2010 or Roche Modular E170</td>
<td>Manufacturer’s reference interval for nonpregnant adults: TSH: 0.27–4.20 mIU/L; 12.0–22.0 pmol/L</td>
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<td></td>
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<tr>
<td>Marwaha et al. (India, 2008)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TSH</td>
<td>0.6–5.0</td>
<td>0.44–5.78</td>
<td>0.74–5.7</td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>12–19.45</td>
<td>9.48–19.58</td>
<td>11.3–17.71</td>
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<tr>
<td>Gong et al. (Canada, 2008)</td>
<td>TSH</td>
<td>11–19</td>
<td>9.7–17.5</td>
<td>8.1–15.3</td>
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<td>ACS:180; Chiron Diagnostics Corporation, East Walpole, MA, USA</td>
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<td>Panesar et al. (Hong Kong, China, 2001)</td>
<td>TSH</td>
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<td>0.03–3.1</td>
<td>0.13–3.5</td>
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<tr>
<td></td>
<td>FT4</td>
<td>11.1–22.9</td>
<td>8.1–16.7</td>
<td>9.1–15.6</td>
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<td>Bayer ADVIA Centaur analyzer</td>
<td>Manufacturer’s reference intervals for nonpregnant adults: TSH: 0.35–5.50 mIU/L, FT4: 11.5–22.7 pmol/L</td>
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<td>Springer et al. (Czech Republic, 2009)</td>
<td>TSH</td>
<td>0.06–3.67</td>
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<td>Yan et al. (China, 2010)</td>
<td>TSH</td>
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<td>0.05–4.50</td>
<td>0.47–4.54</td>
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<tr>
<td></td>
<td>FT4</td>
<td>11.8–21.0</td>
<td>10.6–17.6</td>
<td>9.2–16.7</td>
</tr>
</tbody>
</table>

TSH, thyrotrophin; FT4, free thyroxine

<sup>a</sup> The referee intervals were calculated as 5th and 98th percentiles
<sup>b</sup> The reference intervals were calculated as 5th and 95th percentiles
Thyroid autoimmunity suppresses thyroid function significantly as evident in our study in the TPO-Ab positive women during all three trimesters of pregnancy. We found that TPO-Ab were positive in 12.5% of pregnant women. The prevalence of TPO-Ab in the pregnant population is comparable to that found in the general female population with a similar age range between 5–15 % (324). We looked at TSH ranges when TPO-Ab positive patients were included into the data sample. Interestingly, women with positive TPO-Ab levels had significantly higher the 97.5 percentiles of TSH in all three trimesters to 2.91, 2.82 and 2.52 mIU/L, respectively, when compared to those with TPO-Ab negativity. Though there were no statistically significant differences found in TSH between trimesters in TPO-Ab positive pregnant women. Thus we excluded TPO-Ab positive women from the assessment of thyroid parameters reference range sample. Consequently, TSH values defining the selected upper centiles were lower (2.72, 2.51, 2.36 mIU/L, respectively) when TPO-Ab positive women had been removed from the reference population. Our data is in agreement with the studies in this concern (136-137, 323). TPO-Ab positive women might be at risk for hypothyroidism, especially those with high-normal TSH concentrations, when reference interval for non-pregnant population is used. Interesting data has been published by Negro et al. (159) where thyroxine–treated women with autoimmune thyroiditis maintained the euthyroid status, while in the untreated group with TPO-Ab positivity FT4 decreased by 30% and TSH levels increased progressively during gestation. Consequently, 19% of the latter women became subclinically hypothyroid at the time of delivery. Guidelines say that women with thyroid autoimmunity who are euthyroid in the early stages of pregnancy are at risk of developing hypothyroidism and should be monitored for elevation of TSH above the normal range and that TPO-Ab is the most useful marker for the prediction of postpartum thyroid dysfunction (1, 14). Overall we could conclude that the immune status of the women influences thyroidal changes throughout the course of gestation and a proper approach of evaluation is needed. The reference interval to rely on carries a special concern on diagnosis and initiation of thyroxine treatment in TPO-Ab positive euthyroid woman as it improves pregnancy outcomes (159). Moreover, the increased incidence of pregnancy loss in pregnant women with TSH levels between 2.5 and 5.0 mIU/L provides evidence to support redefining the TSH upper limit of normal to 2.5 mIU/L in the first trimester (164).

Serum FT4 concentrations also have a characteristic pattern during normal pregnancy. This pattern includes a slight and temporary rise in FT4 concentrations during the first trimester and a tendency towards progressive decline in FT4 concentrations during later gestation (82). This pattern was
observed in our study as well as other recent publications (323). This may be associated with an increase in serum thyroid-binding globulin concentrations during pregnancy (325) and with iodine deficiency (174). In our FT4 results showed significant variation between trimesters with values decreasing from first to second trimester, but thereafter remaining, similar to those reported by other studies. (26, 320, 326) Conversely, study in pregnant women in the iodine-deficient area showed an absence of the usual FT4 spike (41). In our study FT4 was not influenced by antibody status, and this was observed in the Switzerland study by Stricker et al. (135).

In addition, FT4 results in pregnancy are influenced by assay performance due to method-dependent artefacts (124) and vary among different assay manufacturers (88, 133, 327), with the results falling in the lower part of the non-pregnant reference interval or below the lower limit (123). Consequently, method specific reference interval is required for FT4 assays (77, 120).

In iodine-sufficient conditions, the physiologic FT4 decrement during the second and third trimester does not exceed 10% (88), while in iodine-deficient nutritional conditions it reaches 20–25% (23). In line with this, Laurberg et al. suggested that a moderate, 10–20% fall in the serum FT4 concentrations during pregnancy is not a sign of maternal dietary iodine deficiency (92). Our study indicates that a borderline 19% decrease in FT4 concentrations provides no clear answer regarding dietary iodine deficiency. A study in Sweden, iodine-sufficient country, has shown the mean FT4 decrease by 15% from the first trimester to the second (134). Our earlier report (328) on TSH concentrations taken on the third day after birth in a sample of 177 newborns from the same cohort of pregnant women established a 2.8% prevalence of increased TSH concentrations above 5 mIU/L, which did not exceed the prevalence rate under iodine-sufficient conditions, which is usually below 3% (329-330), neither indicated dietary iodine deficiency in this sample, nor did maternal TSH profiles in our study. In iodine deficient areas increases in TSH concentrations during late pregnancy have been reported (26, 92).

Lithuania was considered a mild iodine deficient region (330) and severe iodine deficiency was previously reported in pregnant women (32-33). Thus there are counties within Lithuania, where iodine deficiency assessed by the prevalence of goiter using different evaluation criteria in schoolchildren is still moderate or severe (192). Data presented by Barzda et al. (331) show newborn screening TSH over 5.1 mIU/L in 4.3–5.3% of cases during 2002–2005, respectively. A slightly lower newborn TSH in our study might be due to inclusion newborns with risk free for maternal thyroid disorders, assessing only maturely born newborns form exclusively one Lithuanian re-
Our findings support the statement that iodide deficiency is not always uniform across a nation (77). Studies in both Europe and the United States suggest that iodide deficiency should be considered more as a "pocket disorder", meaning that it can be more prevalent in some areas of a country compared with others (21, 191). In 2003 the governmental recommendations for the use of iodized salt were introduced in Lithuania and since 2005 are mandatory according to the Ministry of Health regulations. Sustained salt iodization maintains an adequate iodine status in all population groups, even in pregnant women despite their increased requirements (332).

Therefore, the consequences of iodization programs should be evaluated precisely in respect to the region within the country as well as reporting reference interval for thyroid function should address this issue.

Our longitudinal cohort study examining the pattern of serum TSH concentrations and FT4 concentrations in healthy, TPO-Ab negative pregnant women in three trimesters of pregnancy, have confirmed that during pregnancy thyroid axis hormone reference ranges differ from referral ranges used in non–pregnant population.

Our study confirms successful iodination in Lithuania. We had no chance to measure iodine urine concentration, no data available on additional vitamin use during pregnancy and this is a limitation of the study as well as relatively small sample-size. On the other hand, the longitudinal design is a strength giving better indication of what occurs in thyroid function during pregnancy and how reference ranges should be constructed. A study from a mildly iodine-deficient area revealed that prolonged iodized salt significantly improves maternal thyroid economy and reduces the risk of maternal thyroid insufficiency during gestation (322). We support the need for gestational age specific reference interval when assessing thyroid function in pregnancy.

4.2. Iodine deficiency assessed by the newborn thyroid stimulating hormone concentrations

We analyzed dry blood spot TSH data from the CH screening program in newborns from Kaunas region to assess iodine deficiency. Four methods are generally recommended for assessment of iodine nutrition in populations: urinary iodine concentration (UI), the goiter rate, serum TSH, and TGB. These indicators are complementary in that UI is a sensitive indicator of recent iodine intake (days) and Tg shows an intermediate response (weeks to months), whereas changes in the goiter rate reflect long–term iodine nutrition (months to years)(3).
Generally, TSH can be used as an indicator of iodine nutrition because serum TSH concentration is determined mainly by the level of circulating thyroid hormone, which in turn reflects iodine intake. TSH is a sensitive indicator of iodine status in the newborn period (198, 318). The newborn thyroid contains less iodine but has higher rates of iodine turnover. Particularly, when iodine supply is low, the maintenance of high iodine turnover requires increased TSH stimulation. Serum TSH concentrations are therefore increased in iodine deficient infants for the first few weeks of life, a condition termed transient newborn hypothyroidism (3). Therefore we used neonatal TSH measures obtained three days after birth in our analysis.

According to the statement by WHO/UNICEF/ICCIDD, we evaluated dry blood spot TSH measures, and found 2.8 percent of newborns being above the 5 mIU/L. In the Table 1.1, a frequency of 3–19.9% indicates a mild iodine deficiency (329). Our data showed that the assessed sample is at the margin of iodine sufficiency, thus being a serious hallmark for healthcare providers, assuring and maintaining the sufficient iodine intake for women during pregnancy. The most recent NHANES survey (2003–2004) reported that UI values are decreasing from 294 to 128 μg/L in women of childbearing age, with 37.2% of them having UI values below 100 μg/L, which suggests mild iodine deficiency (333).

It is known that iodine deficiency affects all stages of human life, however, pregnant women, lactating women, women of reproductive age, and children younger than 3 years are considered to be at high risk (334). According to the data of 1995, Lithuania is in the low iodine deficiency area (48, 330) with 22% of pregnant women in 2002 having a severe iodine deficiency (32). Therefore data on the neonatal thyroid screening appear to be particularly interesting in the monitoring of iodine supply at a population level. The data presented by Barzda et al. (207) demonstrated 4.3–5.3% of cases of newborn screening TSH over 5.1 mIU/L during 2002–2005. A slightly lower TSH in our study might be due to inclusion newborns with risk free for maternal thyroid disorders, assessing only maturely born newborns from exclusively one Lithuanian region.

The study by Mickuviene et al. (192) assessed the prevalence of goiter in school-age children (7–11 years) and found that the mean thyroid volume exceeded 97th percentile in 72–82% (counted by M.B. Zimmermann (335)), alerting severe iodine deficiency northeast of Lithuania. According to WHO, a total number of children with goiter exceeding 30%, represents severe iodine deficiency in population (43). Studies in Lithuania are needed to assess the relationship between the iodine status of schoolchildren and the iodine status of pregnant women in the same location, as school-age children, the usual indicator group, are the most appropriate group to reflect the
iodine status of pregnant women (203), especially evaluating consequences of long run mandatory USI program in Lithuania. The national study on the use of iodized salt in Lithuania was undertaken during August – October 2005 by the National Nutrition Center and the results indicated that 67.8% of households were using iodized salt. An improvement over previous studies was seen, e.g. less than 10% of households used iodized salt in 1998, which increased to 39% in 2002 (336). Iodine deficient countries especially, should monitor the state of their iodine nutrition every three years and report to the World Health Assembly on their progress (95).

The limitation of our study is that we cannot currently provide the data on iodine in urine of pregnant women as well as their multivitamin pill use during pregnancy. Nevertheless, we suppose, that women participating in our study could have been self-motivated towards appropriate iodine use, since they knew the aim of the study. We propose that the local situation should be correctly evaluated before starting on medical recommendations for adequate iodine supplementation programs during pregnancy. Finally, healthcare providers should be cautious recommending certain brands of multivitamins for pregnant women as it may not contain all the iodine they claim. Multivitamins contain levels of iodine that are discordant by 50% stated on their label (337). Moreover, there is a final general epidemiological concept that iodine deficiency requires constant monitoring, even after the implementation of iodine supplementation in pregnant women (42, 338).

4.3. The newborn thyroid stimulating hormone levels in relation to maternal age, gestation, birth weight and gender

As it was mentioned above, multiple factors can influence measurements of TSH concentrations in newborns. We observed that older maternal age in pregnancy and newborn gestational age were associated with elevated newborn bloodspot TSH levels on the third day of life. Therefore, a careful consideration of these factors should be drawn while assessing blood spot TSH levels and providing antenatal care.

There is scanty research data available on the dynamics of the newborn thyroid function during the prenatal period and the factors potentially influencing newborn TSH levels. Our data on the relation between newborn TSH levels and maternal age is in close agreement with the study by Herbstman et al. where older maternal age was independently associated with lower umbilical cord total T4 level; nevertheless they did not show the same association with umbilical cord serum TSH levels in multivariate analysis (214). We can only speculate on different study design used as well as different
geographical region and nutrition environment of the newborns and their mothers studied. We can also hypothesize that cord blood TSH levels could be influenced by the wide range of delivery factors.

It is noted that maternal age is increasing among pregnant Lithuanian women. According to statistics of 2001 and 2008, the proportion of delivering women over 30 years of age was 30.9 and 37.7 percent, respectively (339). In our study 31.1 percent of delivering women were after 30 years of age. Older pregnant women have a higher risk of having a baby with a genetic abnormality, such as Down's syndrome, Edwards' syndrome or Patau's syndrome (340). According to the worldwide database, The International Clearinghouse for Birth Defects (341) and EUROCAT (342) collecting information on infants born with congenital malformations, and Italian study from the Italian Registry for Congenital Hypothyroidism (343) show that anomalies of the heart, nervous system, eyes (representing precocious structures in the developing embryo) and multiple congenital malformations are significantly associated with CH (209, 344-345). These findings strongly suggest a very early impairment in the first stages of embryo development with a consequent involvement of different organs and structures. As the fetus progresses into the third trimester, it develops the ability to produce its own thyroid hormones but it is still dependent on maternal iodine for hormone synthesis (80, 238). While most women in Europe are classified as iodine deficient during pregnancy (achieving only approximately half of the recommended daily iodine intake), only 13–50% receive iodine supplementation (346). Therefore, we presume that maternal age and elevated neonatal TSH levels acting jointly might have a significant impact on fetal intrauterine development and hormonal status of the newborn. There are known benefits of folic acid supplementation three months before and during early stages of pregnancy in terms of preventing neural tube defects alone and reducing the incidence of other birth abnormalities such as congenital heart disease, urinary tract problems, oral facial clefts, limb defects, and some others (347-348). On the other hand, there are recommendations that women should increase their daily iodine intake during pregnancy and breastfeeding (1, 31, 190).

In our study we observed that gestational age was associated with increased bloodspot TSH levels in multivariate but not univariate models. In agreement with our study, the relationship between gestational age and TSH concentrations was shown by Korada et al. but multiple regression analysis confirmed that this was a reflection of the close link between gestational age and birth weight (215). On the contrary, Herbstman et al. concluded that gestational age was independently associated with lower cord TSH, higher cord total T4, and higher neonatal and subsequent bloodspot total T4 (214).
In the study by Miyamoto et al. the TSH levels varied widely and had no correlation with gestational age because they were affected by the mode of delivery (225). It is known, that as gestational age increases, the fetus increases the synthesis of both T4 and TSH (238). That statement was confirmed in the study by McElduff et al., where higher TSH values had been associated with older gestational age (200). Studies of fetal and neonatal thyroid function show that thyroid hormone levels rise as pregnancy advances (195, 349) with levels of TSH in cord blood and neonatal blood spot samples positively related to gestational age (214).

In our study, the distribution of TSH was not significantly different between males and females, with males tended to have a higher median blood spot TSH. Theoretically, estrogens and testosterone have the scanty direct effects on TSH synthesis and secretion in humans (350). Nevertheless, similar observation on correlation between gender and bloodspot TSH was made by Korada et al. (215). The studies by Herbstman et al. (214) and Chan et al. (351) found the positive association between elevation of cord blood TSH and male gender, but other researchers (195, 308) did not find significant difference in TSH levels between males and females. Interestingly, a study in Greece (352) assessed thyroid hormones in schoolchildren and found that girls had lower TSH values than boys at puberty. The data on hypothyroidism and infertility presents (353) that hypothyroidism influences ovarian function by decreasing levels of sex-hormone-binding globulin, thereby increasing the number of bioavailable androgens (354). By this approach, we may hypothetically presume that possibly gender specific hormones have an impact on the pituitary-thyroid axis and TSH regulation, therefore male gender expressing higher TSH values.

Our data revealed that gestational age and maternal age were the strongest predictors of newborn TSH on the third day of life. We suppose health care providers to be cautious providing antenatal care to the older woman in terms of fetal and neonatal thyroid function, specially, when there is no consensus on universal screening for hypothyroidism in pregnancy (1). A case finding approach is recommended (1) in women at high risk for thyroid dysfunction, but maternal age is not included in the criteria. Moreover, there is a recent report on screening for CH and defining upper threshold limit for newborn TSH (217), thus evaluating maternal age and neonatal TSH associations, we propose to be alert while interpreting newborns TSH during the first days postpartum, born to older pregnant women. We suppose maternal age factor could also be taken into account while deciding on iodine supplementation during pregnancy as well.
4.4. Delivery mode impact on newborn thyroid stimulating hormone concentrations

We measured blood spot levels of TSH in newborns delivered by mothers who had no thyroid disorders, and did not find significant differences of TSH concentration in newborns delivered vaginally, by vacuum extraction or cesarean section. The same was concluded by other researchers (308, 318). Nevertheless, we have found diverse data on newborn thyroid function effects and the delivery mode.

Few studies confirmed that TSH levels were higher in newborns born by cesarean section (199)(193-194). Interestingly, in the study by McEllduff et al, babies delivered by cesarean section were significantly more likely to have TSH levels greater than 5 mIU/L on the third day of life than those delivered vaginally (200). Moreover, the incidence of false positive TSH tests at screening (> or =15.0 mIU/L) was higher in newborns born by cesarean section (351). With the rise in the rate of births by cesarean, this could be an important factor in assessing population iodine deficiency using neonatal TSH levels. One mechanism to explain this stimulatory effect on newborn TSH is that topical iodine skin preparation for cesarean section deliveries has an iodine load to the mother, part of which is transferred to the infant resulting in acute inhibition of thyroid function (Wolff-Chaikoff effect). A similar, but more marked elevation in neonatal TSH has been documented with topical iodine antiseptic use on the newborn in some (311-314), but not all reports (315-316).

In contrast, the results by other studies (190-192, 301) reported higher cord blood TSH concentrations in infants born after normal vaginal delivery than newborns born by caesarean section, although Miyamoto et al. (225) reported that this did not persist to interfere with congenital hypothyroidism screening. Thus, TSH levels tended to be higher in the vaginal delivery group compared to the elective cesarean section group (355). Our data is in agreement with the above researchers, by stating that mean serum TSH level following normal vaginal delivery was higher than elective cesarean section (but not significantly). Moreover, emergency cesarean section tended to show higher newborn TSH levels than elective cesarean section. We can only suppose that delivery stress was lowest in born by elective cesarean section, thereby, presented newborn lower TSH could be stress related. Moreover, similar parallel could be drawn between newborn TSH, Apgar scores and delivery stress, discussed later on.

The mean cord serum TSH level in newborns delivered by vacuum extraction was significantly higher than the level following normal vaginal delivery (191, 319) or caesarean section (356). However, the mean TSH levels...
of newborns on the third day in heel blood spotted filter paper had no significant differences among normal vaginal delivery, vacuum extraction and caesarean section (356).

We also observed Apgar scores at 5 minutes effect on TSH. Infants with Apgar of ≤8 at 5 minutes had higher mean levels of TSH as compared to those with Apgar of 9–10 at 5 minutes, in consistent with the findings by Herbstman (214). Additionally, it has been reported by other studies that the Apgar score at 1 and 5 minutes is inversely associated with cord blood and bloodspot TSH (197-198). Moreover, infants with asphyxia at birth (Apgar score <4 at 5 min) had significantly higher cord blood TSH levels as compared to those without (228). Such findings rise the hypothesis that perinatal-delivery stress might have an impact on TSH elevation.

We reported for the first time dry blood spot TSH measures in association with some delivery data in the sample of newborns delivered by the thyroid disease wise healthy Lithuanian women during pregnancy, that now can be compared to the similarly designed studies. Although, the mode of delivery have not had considerable influence on TSH in the newborns might be due to a small sample size, but minor differences exist. We suggest that the mode of delivery should be taken into consideration while interpreting newborn blood spot TSH results.

4.5. Antenatal maternal mental state and anthropometric characteristics of newborns

Our present study demonstrates the strongest predictors altering newborn anthropometric parameters in relation to certain psychological factors, as well as maternal thyroid hormones throughout different time points in pregnancy.

Firstly, we assessed maternal thyroid axis relation to newborn TSH. We found that median newborn blood spot TSH concentrations were slightly higher than the maternal values. As mentioned before, perinatal stress, environment temperature changes might have influenced the difference in values. It is noticed that throughout gestation, the serum TSH values are greater in fetus than are present in maternal circulation and higher than would be expected in adults with normal thyroid function (45). Moreover, we did not find correlations between maternal thyroid axis hormones in all trimesters of pregnancy. This finding suggests that the development and function of the fetal thyroid depends on TSH stimulation from the fetal pituitary itself and is unrelated to maternal pituitary stimulation. As our maternal thyroid axis hormones were assessed relatively late in gestation, starting from 12th
week, there remains a question on maternal-fetal regulations early in gestation. However, in the study performed in marginally low iodine deficiency area, mean newborn TSH level was found to be significantly higher than maternal values, but newborn TSH levels increased in parallel and were highly correlated with maternal data, suggesting a regulatory link between both thyroid economies (357). Presence of thyroid antibodies in euthyroid women is associated with miscarriage (157), thus we looked at associations of TPO-Ab and newborn thyroid. We found no evidence that elevated TPO-Ab would be associated with newborn TSH concentrations, in a way confirming the fetal immune tolerance to maternal factors (286).

Secondly, we analysed maternal anthropometric measurements in relation to newborn anthropology. Our study found a positive relation between maternal weight, height, BMI and newborn birthweight and height. A positive relationship between maternal BMI and the birth weight of the offspring was also found in the other studies (358-360). As it was expected, gestational age was strongly associated with newborns birthweight, height and BMI. Pregestational maternal body size indices and weight gain during pregnancy have repeatedly been shown to be independent determinants of the size of the offspring (253-254). We found that maternal pre-pregnancy weight has the positive effect on newborn birthweight in multiple regression analysis. Studies that reported pre-pregnancy weight and height found a significant association with either low birth weight (less than 2500 g) or the birthweight (361-363). This association is broadly addressed in the countries with socio-economic deprivation (68). Poor maternal weight status is a risk factor for growth restriction and low weight births and adverse pregnancy outcomes (364-365). On the other hand, maternal body weight gain during pregnancy is associated with increased birth weight of the offspring (366), greater incidence of pregnancy complications (367-368), the greater weight gain, the greater risks (369). We also analyzed maternal weight gain during pregnancy and found it to be a strong positive predictor of newborn height. Having in mind that maternal prenatal obesity in developed countries has increased significantly over the past 15 years with maternal body weight at the first prenatal visit increased by 20% (370-371), attention should be drawn to the consequences of the latter. Moreover, several recent publications have demonstrated that newborn and infant body weight and BMI are important factors predicting cardiovascular morbidity, stroke and diabetes in adults (372-375). Recent gestational weight gain recommendations are based on a woman's pre-pregnancy BMI (376). Entering pregnancy with a normal BMI and gaining within the recommended gestational weight gain ranges minimizes maternal, fetal and newborn risks. Thus, maternal pre-
pregnancy weight, weight gain should be an important family planning issue and counsel of healthcare specialist on proper nutrition during pregnancy.

In our study higher FT4 concentration at second trimester of pregnancy predicted lower birthweight and lower BMI of the newborn. Multiple retrospective studies have reported an association of poorly controlled hyperthyroidism with intrauterine growth restriction or low birth weight compared with treated euthyroid women (20, 377-378). More specifically, hyperthyroidism in the third trimester was an independent risk factor for low birth weight (379). Similar findings were observed in the first trimester, were hypothyroxinemia was associated with preterm labor and macrosomia (380). Interestingly, studies in adults suggest that low normal FT4 is associated with higher BMI (381) and obesity (382), as well as overt hypothyroidism in adults is associated with weight gain, while hyperthyroidism is associated with weight loss (383).

Moreover, we found in the multiple regression analysis that FT4 concentration in the third trimester of pregnancy was the strong negative predictor of birth weight and together with other significant maternal psychological factors covered 31% of variance. The study by other researchers, assessing UI found that newborns had lower mean birthweight with UI below 50 μg/L in the third trimester of pregnancy (384), but they did not find association between FT4 and TSH measured during the first trimester (384).

Moreover, thyroid hormone changes during pregnancy were noticed to have an impact on psychological factors (60, 248). We found 9.8% women in the first, 4.6% in the second and 6.9% women in the third trimester of pregnancy with depressive symptoms, which is in agreement with a recent review of the literature (385). Recently published ACOG opinion reflects the importance of evaluation of depressive symptoms in pregnancy. Although the organization noted that currently, there is insufficient evidence to support a firm recommendation for antepartum or postpartum screening, evaluating patients for, diagnosing, and treating depression, it has the potential to benefit a woman and her family and should be strongly considered (62). As a result of our research, we determined that 11.6% of women experienced depressive symptoms at least in one assessment throughout three trimesters of pregnancy. Leaving an open quest for discussion on the usefulness of screening for depression symptoms antenataly, more so as the EDS is easy to fill-in and not time consuming instrument, giving important information on the psychological state of the childbearing women.

We looked at maternal depression as the possible predictor of newborn anthropometry, since often undetected and uncontrolled maternal depression in pregnancy puts the developing fetus at harm due to consumption of alcoholic beverages and smoking during pregnancy (386-387), poor prenatal
care (388) and suicide attempts (389), including behavioral problems later in childhood (277). Furthermore, depression occurrence during pregnancy poses further risks to women (390) as it is associated with obstetric complications, such as miscarriage, premature delivery, premature rupture of membranes, preeclampsia, postpartum depression (263, 391-393) and newborn birthweight and body mass index alternations (64, 269, 394-396).

We observed that higher EPDS score in the first trimester of pregnancy was a strong predictor together with other cofounders of higher newborn weight at birth and BMI. In contrast, a recent study in the developing country (Pakistan) shows that depression in the third trimester of pregnancy predicts low birth weight (394). Whereas, Andersson et al. (397) in their Swedish and Berle et al. (398) in their Norwegian large population-based studies on maternal antenatal depressive disorders and/or antenatal anxiety disorders did not find any differences in newborn birthweight. Similarly, Larsson et al. did not find associations between antenatal depression low birthweight (399).

Contradictions between the findings in the studies can be explained by methodological differences, where assessment of depression and anxiety was made in different trimesters of pregnancy by different self-assessment questionnaires and even for the same scales different cut-off points were applied. Furthermore, contrasting findings in the studies could be explained by cultural differences and coping with depression under certain socio-economical conditions which predispose certain behaviors of women, which may lead to adverse pregnancy outcomes.

It is known that some emotional, psychological responses, such as depression, anger, anxiety might be mediated by stress. In our study we looked at acute and chronic stress impact on newborn anthropometry, as stress has profoundly harmful effects on reproduction, particularly during gestation, and is causally associated with pregnancy failure (400) and adverse development of offspring health (401-402), including craniofacial malformations and heart defects (403).

Stress increases hormone secretion from the euroendocrine HPA axis. Psychosocial stress has been shown to be associated with elevated maternal cortisol levels during pregnancy (404-405), thus there are several possible mechanisms how signals of maternal stress may reach the fetus. Firstly, by reduction in blood flow to the uterus and fetus at increased levels of maternal stress (281). Secondly, by transplacental transport of maternal hormones (406). Thirdly, by stress-induced release of placental corticotrophin-releasing hormone to the intrauterine environment (407). All of the mentioned factors restrict the delivery of oxygen and nutrient to the fetus, subsequently, affecting fetal growth and development (408).
We analysed acute and chronic stress exposure impact on anthropometrical measures during different gestational time periods and found that chronic stress in the second trimester of pregnancy was positively associated with birthweight. Interestingly, when we conducted multivariate regression together with FT4 and other factors, we found that both chronic stress and FT4 in the third trimester of pregnancy predicted lower birthweight of the newborn. Although, maternal chronic stress in the second and third trimester of pregnancy has a divergent impact on newborn weight at birth, we suppose, it could be partially explained by the mentioned above possible mechanisms how signals of maternal stress may reach the fetus. The time window of the influence of maternal stress induced cortisol secretion on fetal growth is however, unknown. While it is suggested that early gestation may be a particular sensitive period for maternal, placental, fetal pathophysiology (408-409), late pregnancy is a critical period as it is known for the accelerated fetal somatic growth (410). Only a few studies focus on the timing effects of life events on birth outcomes (411-414). However, when the women are exposed to chronic stress, they fail to show an up-regulation of placental enzyme activity converting cortisol to the inactive form, cortisone, indicating that chronic stress may be detrimental for healthy pregnancy (401, 415). In addition, Hobel et al. have reported that maternal stress at 18–20 weeks of gestation predicted a rise in corticotropin-releasing hormone levels at 28–30 weeks of gestation (416). Our findings show that chronic stress at 22–26 weeks of gestation positively effects fetal growth, but later on, in pregnancy at 32–36 weeks of gestation has an opposite action. Summarizing all above, we could hypothesize that our findings support the stress related data on maternal placental fetal pathophysiology on the clinical level, predicting newborns weight.

Moreover, our results show chronic stress impact on the newborn anthropometry that could be compared with the studies on cortisol and neonatal outcomes. Previous studies observed an association between maternal cortisol levels and fetal growth (417), estimated fetal weight (418-419) or birthweight (410, 418). Thus, a recent study in Netherland did not observe a mediation effect of maternal cortisol on the association between maternal psychosocial problems and offspring birthweight (420). In the other studies psychosocial stress (421) and adverse life events (422) were not risk factors for low birthweight newborns. Although Newton et al. (423) reported a significant association between life events and low birthweight, they did not control for confounding variables.

Our study revealed an impact of acute stress in the second trimester of pregnancy on the birthweight of the newborn. Interestingly, we noticed that chronic and acute stress in the second trimester of pregnancy had a positive
effect on birthweight, whereas chronic stress in the third trimester has a negative effect on newborn birthweight. Other studies showed that exposure to acute stress in the first trimester (424), but not in the second and third trimesters, had a greater negative effect on the birthweight of newborn (414, 425).

We suppose that the most vulnerable period for the effects of maternal chronic stress and thyroid hormone actions appears to be in the third trimester, when the fetal growth spurt occurs. Nevertheless, attention should be drawn to the assessment of exposure to chronic stress during early and late pregnancy, especially knowing that it precipitates long-term adverse consequences on offspring (e.g. cardiovascular disease, depression) (285).

The degree of stress response depends also on genetic factors, personality characteristics, previous experience, support from the social environment, and the way of coping with stress (265). This applies to pregnant women as well (265). We evaluated five maternal personality traits and found that personality dimension was not associated with both perceived acute or chronic stress. Though, we observed that conscientiousness was negatively related to depression and anxiety symptomology during pregnancy. Similarly to others, conscientiousness was inversely related to major depressive disorder, but different from us, demonstrated a positive relationship with generalized anxiety disorder (426). Moreover, in our study neuroticism was strongly related to anxiety and moderately to depression. Extraversion was inversely associated with anxiety during pregnancy. The etiological trait approach assumes that personality is an independent risk factor for disease. This means that various early biological characteristics of the individual are related to both personality and health (427).

Therefore, we evaluated maternal personality dimensions impact on the newborn anthropometrical measurements. We found that only one, namely, conscientiousness, was revealed regardless of positive effect on newborn birthweight in the second pregnancy trimester. This significant association persisted after adjusting for maternal age in multivariable analysis. Hereby, we suppose, that maternal conscientiousness, reflecting dependability, such as being careful, thorough, responsible, organised and planful, could potentiate a certain behavioral pattern during pregnancy that favors newborn anthropometrical measures.

In summary, our study demonstrates that maternal anthropometrical measurements, psychological factors and thyroid function acting jointly are associated with birth outcome, namely, anthropometrical measurements of the newborns. The protective effect of personality trait - conscientiousness, was observed, as well as the negative effect of the perceived chronic psychosocial stress and elevated free thyroxine in the third trimester of preg-
nancy, regardless of the birth weight. Therefore, combining different areas, such as maternal mental health, nutrition, hormonal balance in pregnant women brings relevant information on the predictors of newborn anthropometrical measures. Health care supervisors should pay attention to woman’s nutritional status before pregnancy, as it is a strong determinant of newborn outcomes. We propose that interventions to prevent or reduce stress, diagnosis of anxiety, depression symptoms or disorder should be started before conception and should be observed and maintained carefully throughout the pregnancy.
CONCLUSIONS

1. Recommended reference intervals for TSH concentrations were found to be 0.02–2.72 mIU/L for the first trimester, 0.22–2.51 mIU/L for the second trimester and 0.28–2.36 mIU/L for the third trimester of pregnancy. Recommended reference intervals for FT4 concentrations were found to be 13.2–23.1 pmol/L for the first trimester, 11.8–18.5 pmol/L for the second trimester and 10.5–18.3 pmol/L for the third trimester of pregnancy. Thyroid autoimmunity assessed by thyroid–peroxidase antibody concentrations increases maternal TSH concentration and have no effect on FT4 during all trimesters of pregnancy.

2. Screening of the iodine deficiency using indirect measure, dry blood spot thyroid stimulating hormone concentrations in newborns, with 2.8 percent newborns being above the 5 mIU/L, showed iodine sufficiency at the implementation of the universal salt iodisation program in Kaunas, Lithuania. A 19% decrease from peak to nadir in FT4 concentrations during pregnancy confirms sufficient dietary iodine intake in pregnant women.

3. We have found that older maternal age at delivery and longer gestation is associated with elevated newborn bloodspot TSH levels, but the mode of delivery has no considerable influence. The third day after birth newborn TSH concentrations failed to show association with maternal thyroid function and autoimmunity.

4. The newborn birth weight and BMI is negatively influenced by higher maternal thyroxine concentration in the second, perceived chronic psychosocial stress in the third trimester of pregnancy, whereas maternal personality trait, conscientiousness, symptoms of antenatal maternal depression in the first and perceived chronic psychosocial stress in the second trimester have positive effect.
SCIENTIFIC SIGNIFICANCE OF THE STUDY

New information of scientific value on psychoendocrine challenge in pregnancy is contained in this study. We have reported for the first time changes in thyroid status and presented the trimester-specific reference intervals for thyroid stimulating hormone and free thyroxine in the sample of healthy Lithuanian women during pregnancy that now can be compared to the similarly designed studies at the time of gestation and different geographical endpoints.

The longitudinal design of the study provides evidence that maternal emotional state and thyroid function, as a psychoendocrine disruptor, found to be associated with birth outcomes of newborn. The results suggest an association between maternal psychosocial stress, personality traits, free thyroxine and newborn anthropometrical measurements. Therefore, combining different areas, such as maternal mental health, nutrition, thyroid hormonal balance in pregnant women brings relevant information on the predictors of newborn anthropometric measures.
PRACTICAL RECOMMENDATIONS

We suggest using trimester-specific reference intervals for thyroid axis function assessment in pregnancy. Reference intervals for TSH concentrations were found to be 0.02–2.72 mIU/L for the first trimester, 0.22–2.51 mIU/L for the second trimester and 0.28–2.36 mIU/L for the third trimester of pregnancy. Reference intervals for FT4 concentrations were found to be 13.2–23.1 pmol/L for the first trimester, 11.8–18.5 pmol/L for the second trimester and 10.5–18.3 pmol/L for the third trimester of pregnancy. We cannot provide evidence in favor of screening for thyroid dysfunction in pregnancy, but may advocate research projects concerning this issue.

Thyroid screening of the newborns showed iodine sufficiency using indirect method of blood spot TSH measures, with 2.8 percent newborns being above the 5 mIU/L. We suppose that the degree of iodine deficiency should be assessed in each concerned area specifically, and the local situation correctly evaluated before starting on medical recommendations for adequate iodine supplementation during pregnancy.

Maternal age, newborns gestational age and delivery mode factors should be carefully considered while interpreting newborn’s blood spot TSH levels.

Health care supervisors should pay attention to woman’s nutritional and thyroid function status throughout pregnancy, as the determinants of newborn outcomes. We propose that interventions to prevent or reduce stress, diagnosis of depression symptoms and anxiety should be started before conception and should be observed and maintained carefully throughout the pregnancy.
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Kаuno Regioninis Biomedicinių Tyrimų Etikos Komitetas

Kauno regioninio biomedicinių tyrimų etikos komiteto posėdžio, įvykario
2002 m. 23 d.
protoko Nr. 8

Kauno regioninis biomedicinių tyrimų etikos komitetas, pirmininkejant doc I. Marchertienėi,
apaštarę mokslinio eksperimento:

"Nešuano ir gimdymo metu patiriamų psichoendokrinių pokyčių įtaka motinos ir vaiko
gerbuvui: multcentrinis tyrimas"

kuris bus atliekamas KMU Akademijos ir ginokologijos klinikose ir KMU Endokrinologijos institutu,
pateiktą medžiagą etinius, medicininiaus, juridinius ir mokslinius aspektus. Komitetas neprieštarauja
experimento vykdymui.

Mokslinio eksperimento vykdytojai įsipareigoja: (1) nedelsiant informuoti Kauno
Regioninį Biomedicinių Tyrimų Ethikos Komitetą apie visas nenumatytes atvejus,
susijusus su studijos vykdymu, (2) iki saujo 15 dienos - pateikti metini studijos vykdymo
apibendrinimą bei, (3) per mėnesį po studijos užbaigimo, pateikti galutinį pranešimą apie
eksperimentą.

Data 2002. 10. 23
Pirminingas I. Marchertienė

Data 2002. 11. 24
Sekretorius R. Juknevičaitė

Data 2002. 11. 24
Studijos vadovas

Data 2002. 10. 23
Studijos vykdytojas

129
Annexe 2

KAUNO REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS
KMUK Eivenos 2, Centrinio korpusas 71, 2007 Kaunas, tel. +370 37 324 901, faks. +370 37 324 901, e-mail: etikos@kausa.lt

PRITARIMAS
PROTOKOLO PTAISOMS / PAKEITIMAMS
2007-06-05 Nr. P1-84/2002

Biomedicinio tyrimo pavyzdinimas: „Nėščiomo ir gimdymo metu patiriamų psychoendokrininių pokyčių įtaka motinos ir vaiko gerbūriui: multicenterinis tyrimas“

Pagrindinis tyrėjas: Habil.dr. Robertas Bunevičius, KMUK endokrinologijos institutas
Prof. Rūta Radišauskienė, KMU akušerijos ir ginekologijos klinika
Doc. Eimantas Švedas, KMU akušerijos ir ginekologijos klinika

Peržiūrėti šie [v] su minėtu tyrimu susiję dokumentai:

[v] Mokslo tyrimo aprašymas;
[v] Papildomų tyrėjų ir pagrindinių tyro įgūdžių gyvenimo aprašymai.

Nutarta:

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Vardas, Pavarde</th>
<th>Veiklos zonė</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Doc. Irena Marchertiene</td>
<td>anesteziologija</td>
</tr>
<tr>
<td>2.</td>
<td>Dr. Romualdas Mešulaitis</td>
<td>klinikų tarptautinė kauplininkų komitetas</td>
</tr>
<tr>
<td>3.</td>
<td>Prof. Nijole Daile Bakšienė</td>
<td>pediatra</td>
</tr>
<tr>
<td>4.</td>
<td>Doc. Teršiškis Norkus</td>
<td>chirurgas</td>
</tr>
<tr>
<td>5.</td>
<td>Daiva Zagaricienė</td>
<td>dieta</td>
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<tr>
<td>6.</td>
<td>Laima Vasiliauskaitė</td>
<td>psychoterapeutė</td>
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<td>7.</td>
<td>Doc. Marija Urbanienė</td>
<td>žemes ūkis</td>
</tr>
<tr>
<td>8.</td>
<td>Egle Vaitikelienė</td>
<td>vadyba</td>
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</table>

Kauno regioniaus biomedicininio tyrimo etikos komiteto nariai

Pirmininkė

Doc. Irena Marchertiene

130
Annexe 3

Gerbiau ponia,
Mes norejome sužinoti, kaip Jūs jaučiote. PABAUKITE atsakymą, kuris artimiausias Jūsų savijautai per pastarąją SEPTYNIAS DIENAS (neapsitikite savijauta šiandien). Čia pateikiamas pavazylys kaip reikia pildyti:

Aš jaučiausi linksma:

Taip, visą laiką
Taip, didžiąją laiko dalį
Ne, nelabai dažnai
Ne, visiškai ne

1. Aš galėjav juoktis ir matyti linksmas gyvenimo pusęs

- Taip kaip visada
- Dabar kiek mažiau
- Dabar žymiai mažiau
- Visiškai ne

2. Aš žvelgiau į ateitį su džiaugsmu.

- Taip kaip visada
- Kiek mažiau nei ankščiau
- Žymiai mažiau nei ankščiau
- Visiškai ne

3. Aš be reikalo kaltinau save, jeigu nepasiekdavo

- Dažniausiai
- Kartais
- Retai
- Niekada

4. Aš jaudavausi be priežasties nerami ir susirūpinusi

- Visiškai ne
- Labai retai
- Kartais
- Labai dažnai

5. Aš be rimto priežasties jaudavausi išsigandusi ar apimta panikos

- Gana dažnai
- Kartais
- Neypatingai
- Visiškai ne

6. Aš negaliu išspręsti kylančių problemų

- Taip, didžiąją laiko dalį aš visiškai nepajęgų į išspręsti
- Taip, kartais aš nepajęgų į išspręsti taip sekmadingai kaip ankščiau
- Ne, didžiąją laiko dalį aš susitvarkau
- Ne, aš susitvarkau kaip visada

7. Aš tokia nelaiminga, kad pradedau blogai miegoti.

- Taip, didžiąją laiko dalį
- Gana dažnai
- Kartais
- Visiškai ne

8. Aš jaučiausi litūna ar suvargusi.

- Taip, didžiąją laiko dalį
- Taip, gana dažnai
- Kartais
- Visiškai ne


- Taip, didžiąją laiko dalį
- Gana dažnai
- Tūkstantais
- Niekada

10. Man kildavo mintys susižaloti.

- Gana dažnai
- Kartais
- Labai retai
- Niekada
### II. Stresogeniniai įvykiai

Perskaitykite žemiau išvardintus stresogeninius gyvenimo įvykius ir pabraukite tuos, kurius patyrėte per pastaruosius 12 mėnesių:

<table>
<thead>
<tr>
<th>Kodus</th>
<th>Įvykiai (trukmė iki 6 mėnesių)</th>
<th>Ilgai trukančios aplinkybės (trukmė daugiau nei 6 mėnesių)</th>
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<tr>
<td>1</td>
<td>Širdies ir kraujos sistemos iškraunsimas</td>
<td>Néra užsitraukusių aplinkybių</td>
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<td>2</td>
<td>Išskyrimas nuo draugo ar drauge</td>
<td>Šeimos ginčai</td>
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<td></td>
<td>Įstojimas į mokytis ar mokslo baigimus</td>
<td>Nepasitenkinimas darbu</td>
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<td></td>
<td>Vaikas paliko namus</td>
<td>Gyvenimas šalia kaimynų, užsiimančių nusikalstama vėkla</td>
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<tr>
<td>3</td>
<td>Vedybos</td>
<td>Sutrukintų nesantaika</td>
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<tr>
<td></td>
<td>Darbo paradimas</td>
<td>Rimtos įrankinės problemas</td>
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<td></td>
<td>Išejimas į pensiją</td>
<td>Nesutarimai su viršininku</td>
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<td></td>
<td>Persileidimas</td>
<td>Vieniša motina ar tėvas</td>
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<td></td>
<td>Sutuoiktinių gyvenimas atskirai</td>
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<td>4</td>
<td>Skrybės</td>
<td>Bedarbystė</td>
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<td></td>
<td>Pirmo vaiko gimimas</td>
<td>Skurdas</td>
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<tr>
<td>5</td>
<td>Sutuoiktinio mirtis</td>
<td>Sunki lėtine paties asmens ar jo vaiko liga</td>
</tr>
<tr>
<td></td>
<td>Sunkios somatines ligos</td>
<td>Besiūnesti fizinė ar seksualinė prievara</td>
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<td>nustatymas</td>
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<td>išžaginimas</td>
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<td>6</td>
<td>Vaiko mirtis</td>
<td>Nelaisvė</td>
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<tr>
<td></td>
<td>Sutuoiktinio savižudybė</td>
<td>Buvimas įkaitu</td>
</tr>
<tr>
<td></td>
<td>Stichinės nelaimės</td>
<td>Buvimas koncentracijos stovykloje</td>
</tr>
</tbody>
</table>
**Spilbergerio STAI–Trait skalė**

Perskaitykite žemiau išvardintus teiginius ir pažymekte Jums tinkamiausią atsakymą (skaičių atsakymų grafoje). Teisingų ar neteisingų atsakymų nėra, todėl ties kiekvienu teiginiu ilgai negalvokite.

<table>
<thead>
<tr>
<th>Eil. Nr.</th>
<th>Teiginys</th>
<th>Atsakymas</th>
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<tbody>
<tr>
<td></td>
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<td>Labai retai</td>
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<td>1.</td>
<td>Aš jaučiu pasitenkinimą</td>
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<tr>
<td>2.</td>
<td>Aš greitai pavargstu</td>
<td>1</td>
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<tr>
<td>3.</td>
<td>Aš lengvai apsiverki</td>
<td>1</td>
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<td>4.</td>
<td>Aš noriu būti toks laimingas (-a) kaip kiti</td>
<td>1</td>
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<tr>
<td>5.</td>
<td>Būna, kad aš pralaimiu todėl, kad negaliu greitai apsispręsti</td>
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<tr>
<td>6.</td>
<td>Aš jaučiuosi žvalus (-i)</td>
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</tr>
<tr>
<td>7.</td>
<td>Aš ramus (-i), šaltakraujiškas (-a), susikaupęs (-usi)</td>
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<td>8.</td>
<td>Sunkumų laukimas man kelia nerimą</td>
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</tr>
<tr>
<td>9.</td>
<td>Aš per daug pergyvenu dėl smulkmenų</td>
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</tr>
<tr>
<td>10</td>
<td>Aš visai laimingas (-a)</td>
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</tr>
<tr>
<td>11</td>
<td>Aš per daug viską imu į širdį</td>
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</tr>
<tr>
<td>12</td>
<td>Man trūksta pasitikėjimo savimi</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Aš jaučiuosi saugiai</td>
<td>1</td>
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<tr>
<td>14</td>
<td>Aš stengiuosi išvengti sunkumų ir kritišką situacijų</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>Mane apima slogi nuotaika – būnu paniurčis (-usi)</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>Aš būnu patenkintas (-a)</td>
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</tr>
<tr>
<td>17</td>
<td>Visokios smulkmenos blaško ir jaudina mane</td>
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</tr>
<tr>
<td>18</td>
<td>Aš taip sunkiai pergyvenu nusivylius, kad po to negaliu jų pamiršti</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>Aš ramus, susitvardantis žmogus</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>Mane apima sunkus nerimas, kai aš galvoju apie savo reikalus ir rūpesčius</td>
<td>1</td>
</tr>
</tbody>
</table>
The Big Five Personality Test

Nurodymai: žemiau yra nurodytos kai kurios savybės, kurios jums gali būti būdingos arba nebūdingos. Pavyzdžiui, ar sutiktumėte su teiginiu, kad esate žmogus mėgstantis leisti laiką su kitais žmonėmis? Prašome prie kiekvieno teiginio parašyti skaičių nurodant, kuria dalimi sutinkate ar nesutinkate su tuo teiginiu.

1 – Visiškai nesutinku
2 – Iš dalies nesutinku
3 – Nei sutinku, nei nesutinku
4 – Iš dalies sutinku
5 – Visiškai sutinku

Manau, kad esu...

1. Kalbus(-i).
2. Linkės(-usi) ieškoti kitų kaltės.
4. Prislėgtas(-a), liūdnas(-a).
5. Originalus(-i), turintis(-i) naujų idėjų.
6. Santūrus(-i).
7. Paslaugus(-i) ir nesavanaudiškas(-a).
8. Kartais kiek nerūpestingas(-a).
9. Atsipalaidavęs(-usi), lengvai susitvarkantis(-i) su stresu.
10. Besidomintis(-i) daugeliu įvairiausių dalykų.
11. Pilnas(-a) energijos.
12. Žmogus, kuris pradeda ginčus su kitais.
13. Patikimas(-a) darbuotojas(-a).
15. Išradingas(-a), giliai mąstantis(-i).
16. Entuziastingas(-a).
17. Atlaidus(-i).
18. Dažnai netvarkingas(-a).
19. Daug nerimaujantis(-i).
20. Turintis(-i) turtingą vaizduotę.
21. Linkęs(-usi) būti tylus(-i).
22. Paprastai pasitikintis(-i) žmonėmis.
23. Linkęs(-usi) į tinginystę.
24. Emociškai stablus(-i), nelengvai nuliūdinamas(-a).
25. Kūrybingas(-a).
26. Ryžtingas(-a).
27. Galiu būti šaltus(-a) ir abejingas(-a).
28. Atkakliai siekiantis(-i) užbaigti pradėtą darbą.
29. Mano nuotaikos gali greitai keistis.
30. Vertinantis(-i) meninius, estetinius išgyvenimus.
31. Kartais drovus(-i) ir suvaržytas(-a).
32. Atidus(-i) ir malonus(-i) beveik visiems.
33. Savo darbus dirbū efektyviai.
34. Įtemptose situacijose išlieku ramus(-i).
35. Teikiu pirmenybę pagal nusistovėjusią tvarką atliekamam darbui.
36. Draugiškas(-a) ir mėgstantis(-i) bendrauti.
37. Kartais grubiai kalbu su kitais.
38. Kuriu planus ir juos įvykdu.
39. Lengvai susinervinantis.
40. Mėgstu galvoti, žaisti idėjomis.
41. Turiu keletą meninių pomėgių.
42. Mėgstu bendradarbiauti su kitais.
43. Mano dėmesys lengvai nukrypta į šalį.
44. Turiu subtilų meninių muzikinį ir literatūrinį skonį.