Chapter 1

General Introduction
Depression is a condition characterised by an enduring sad mood, an inability to experience pleasure and concomitant a lack of interest in, and motivation for, almost all activities. This condition is often accompanied by disturbances of sleep, appetite, energy level and cognitive functioning. The clinical presentation of depression according to the current definition (i.e. based on DSM-IV) is heterogeneous with regard to its symptoms, course and reaction to treatment. This heterogeneous character of depression may cause inconsistencies in the results of studies investigating the causes of depression. Because of this, in most studies homogeneous cohorts are included not reflecting the heterogeneity seen in clinical practice. For research to be more beneficial to actual clinical practice, it might be of use to disentangle the vague concept of depression and investigate more specific and better defined entities, like the development or recovery of specific symptoms and the severity of symptoms.

Over the last 50 years several biological processes have been found to be associated with depression. The dominant biological theories state that functioning of several neurotransmitters, endocrine systems and genetic factors in interaction with stressful life events cause depression. However, most findings supporting this theory are cross-sectional and it remains to be clarified whether the biological disturbances found in depressed patients are causally related to depression or have to be considered as state-, or trait-markers of depression.

This thesis includes a number of studies aimed at elucidating processes involved in the development of depression or depressive symptoms during the peripartum, i.e. pregnancy, delivery and the postpartum period. The peripartum period was chosen to study depression-related symptoms for several reasons. Firstly, during the peripartum period, hormone levels of oestradiol, progesterone and cortisol undergo marked changes. During pregnancy, levels of these hormones increase dramatically, whereas following delivery they decrease to pre-pregnancy levels in a very short period of time. This phenomenon enables studies investigating whether the increase of hormone levels as such is related to affective instability or, alternatively, whether the rapid change of the hormone levels causes changes in mood. Secondly, during the peripartum period the metabolism of biogenic amines, like serotonin, changes markedly. Malfunction of these biogenic amines has often been associated with the development of depressive symptoms and with the onset of major depressive disorders. Women experience pregnancy, delivery and the postpartum period as a life-event, but the subjective impact differs from person to person. Finally, the peripartum period is characterized by
instability of affect, in particular during pregnancy and following delivery (post partum blues).

These considerations lead us to the theme of the present thesis: how do biological factors influence the course of affective symptoms during the peripartum? The effects of peripheral markers of biogenic amines, including some gene variants, on the course and severity of depressive symptoms were explored. In addition, stress associated hormones, such as noradrenalin and cortisol were studied. Finally, we explored whether long chain poly-unsaturated fatty acids modify the subjective perception and thereby the affective reaction to the peripartum experience. Our studies were performed in a sample of women with relatively uncomplicated pregnancies and in subjects with severely complicated pregnancies. In an animal experiment, using a model mimicking the endocrine events of pregnancy, we tested whether gradual or sudden decline of oestradiol and progesterone can cause increased responses to stress.

In the first paragraphs of the introduction, the concept of depression, its current definition and epidemiology will be introduced, followed by a concise description of some of the biological theories regarding depression. Similarities and differences between the physiology of pregnancy and the suggested pathophysiology of depression will be explicated, and the state of the art of current research on the relation pregnancy and depression will be summarized. Finally, the aims and outline of this thesis will be presented.

**Depression**

A sad mood or an inability to experience pleasure can be normal reactions to bereavement, loss and somatic illness (1, 2). These emotions are considered pathological when they do not remit when the external cause dissipates, or when the intensity of the symptoms is too severe or disproportional to their cause. In a depressed condition people may neglect their personal hygiene and environment and problems may arise with functioning in relationships and work. A severe depressive state can lead to life-threatening situations due to malnutrition, severely neglected self-care and personal hygiene and, in extreme cases to suicide.

There is no ‘natural’ distinction between normal sadness and severe depressive states. Large epidemiological studies illustrate that the number and duration of depressive disorder-related symptoms show a normal distribution in the general population (3) and that the number of symptoms is concomitant with the level of functioning. Consequently the distinction between normal sadness and pathological sadness is arbitrary, and based on measures like the number, severity, and duration of depressive
symptoms and their interference with normal functioning. Currently, the most frequently used definition of depression world-wide is provided by the American Psychiatric Association (APA) and is formulated in the 4th edition of the Diagnostic and Statistic Manual (DSM IV) (4). According to this definition a person must have experienced (a) a depressed mood or (b) loss of interest for two weeks or more for most of the time of the day and at least four of the following symptoms: (c) considerable changes in weight or appetite; (d) insomnia/ hypersomnia; (e) psychomotor agitation or retardation; (f) feelings of fatigue or diminished energy; (g) thoughts of worthlessness and guilt; (h) reduced ability to think and concentrate; (i) frequent thoughts about death and suicide (table 1.). As is implicated by the diagnostic criteria, depression has a heterogeneous symptomatology (5). The duration must be at least two weeks, but

The incidence of depression in the Netherlands, according to the DSM-IV criteria, is six percent in the population younger than 65 years (17). Based on this incidence, it has been calculated that one in seven people experience a depressive episode in his/her life (18). The world-wide life time prevalence ranged from 3% in Japan up to 16.9% in USA (19). The consequences of depression for both the quality of life of patients and their

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<tr>
<th>A.</th>
<th>Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.</th>
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<td><strong>Note:</strong> Do not include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations</td>
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<td>(1)Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).</td>
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<td>(2)Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).</td>
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<td>(3)Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.</td>
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<td>(4)Insomnia or hypersomnia nearly every day.</td>
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<td>(5)Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).</td>
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<td>(6)Fatigue or loss of energy nearly every day.</td>
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<td>(7)Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).</td>
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<td>(8)Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).</td>
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<td>(9)Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.</td>
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<th>B.</th>
<th>The symptoms do not meet criteria for a Mixed Episode.</th>
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| C. | The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning. |

| D. | The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism). |

| E. | The symptoms are not better accounted for by Bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation. |

**Table 1. Diagnostic criteria for depression according to the DSM IV.**
families, as well as the costs for society, are significant (20, 21). These costs can be expressed in disability adjusted life years (DALY’s), which are the sum of the years of life lost due to premature mortality (YLL) and the years lost due to disability (YLD) for incident cases of depression (www.who.int). The summed DALY’s for depression in the Netherlands comprise 113 000 per year (18) and are ranked 4th on the list of DALY’s in the Netherlands, preceded only by coronary artery diseases, anxiety disorders and stroke.

The etiology of depression
Explanations regarding the pathogenesis of depression include hypotheses involving every level of human functioning, from molecular to sociological. A small list; socio-economic status, gender, attachment to parents, parenting style, family structure, personality characteristics and coping style, social support, genetic and epigenetic factors, life-style and dietary aspects, life-events, somatic disease, neurodevelopmental aspects, memory functioning, dysfunction of endocrine systems, neuro-anatomical characteristics, functioning of the biological clock, seasonal aspects, disturbances of the immune system, brain metabolism, cell division, migration synaptogenesis of neurons. Evidence for all of these theories has been provided, but none of these hypotheses has been able to solely, fully explain the pathogenesis, course and symptomatology of depression. This introduction is not the place to extensively discuss all possible etiological theories of depression. However, it is important to keep in mind that depression can be explained from many perspectives, indicating that uni-dimensional theories do not fit the complex clinical reality of depression.

The focus in this thesis will be on some of the currently dominant biological theories about the etiology of depression: 1) the mono-amine hypothesis, 2) the role of stress and the regulation of the stress system, i.e. the Hypothalamus-, Pituitary-, Adrenal-axis (HPA-axis) and its major hormone cortisol; 3) genetic factors. In addition, the influence of the female gonadal hormones estrogen and progesterone and the role of omega-3 fatty acids will be investigated. The background of these theories will be briefly introduced below.
The mono-amine hypothesis

The mono-amine hypothesis was introduced in 1965 by Schildkraut and Bunny & Davis (22, 23) after the reports about the mood elevating properties of the tuberculosis drug ipronazid, and the depressogenic properties of reserpine. Ipronazid inhibits mono-amine-oxydase-A (MAOA), an enzyme degrading the mono-amines serotonin and norepinephrine. Inhibition of MAOA function results in an increased availability of mono-aamines. The other way round, reserpine causes a depletion of the mono-aminergic stores. Consequently it was postulated that a deficiency of catecholamines, mainly norepinephrine, is involved in the precipitation of depression. The emphasis on serotonin came a few years later (24). These findings boosted the research investigating monoamine functioning in depression. Observational studies showed a decrease in mono-amine metabolism in depression as investigated in blood (25), platelets (26, 27), cerebrospinal fluid (24, 28), urine (29, 30), with experimental challenges of mono-aminergic systems (31), post mortem brain research (32, 33) and later MRI and PET-imaging of the brain (34, 35). And pharmacologists developed antidepressant drugs affecting the mono-aminergic system. This mainly led to the development of safer drugs with less side-effects focusing on the serotonergic system (36), starting with tricyclic antidepressants (TCA), followed by the more specific serotonin re-uptake inhibitors (SSRI’s), and finally to the newer, less specific, drugs venlafaxine, mirtazapine and buproprion.

The monoamine theory has dominated psychiatry for four decades, but is strongly criticized over the past years. Reasons for this are the moderate effects of the mono-aminergic drugs which have a delayed onset of action (2-8 weeks), and a moderate clinical response (8, 37). These disappointing effects of mono-aminergic drugs contradict the findings of differences in mono-aminergic metabolism between depressed and healthy persons. This raises the question whether differences in mono-aminergic functioning are causally linked to depression or have to be considered as state markers or vulnerability factors for depression. The (direction of the) causality of the relation between mono-aminergic function and mood can be partly elucidated by prospective psychiatric investigations in situations characterized by a chronic low mono-amine function. The physiology of the serotonergic system facilitates such investigations, as explained in the following paragraphs.

The serotonergic system

Most central serotonergic cell groups are located along the midline of the brain stem in the raphe nuclei, and project to the whole forebrain, limbic system and spinal cord figure 1. Serotonergic pathways have an important regulatory role in hypothalamic,
cardiovascular and thermoregulatory control and modulate the responsiveness of cortical neurons (38). Among the behaviors controlled by serotonin functions are arousal (sleep and wakefulness), feeding behavior, mood regulation and behavioral control (39).

Serotonin is synthesized from the essential amino acid L-tryptophan in two enzymatic steps, first by tryptophan hydroxylase followed by the vitamin B6 dependent aromatic amino acid decarboxylase. Serotonin is degraded to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine-oxydase enzymes (figure 2).

As serotonin cannot pass the blood brain barrier (BBB), cerebral availability depends on the production in central serotonin containing neurons. In physiological circumstances the central tryptophan hydroxylase is unsaturated; therefore the rate limiting step in central serotonin production is the availability of tryptophan. Tryptophan is actively transported over the BBB for which it competes with the other Large Neutral Amino Acids (LNAA) phenylalanine, tyrosine, threonine, leucine, isoleucine and valine. The central availability of tryptophan is therefore a function of the affinity for binding the transporter and concentration of the LNAA’s (40). Tryptophan can be metabolized in a second pathway by the enzyme indoleamine-2,3-di-oxygenase (IDO) which will leads to the formation of kynurenine, as shown in figure 2.

In conclusion, the serotonergic system modulates a wide range of neural functions, and consequently behaviors (39). The production of serotonin depends on the central availability of tryptophan. Therefore, naturally occurring (41), or experimentally induced (31) changes in plasma tryptophan or LNAA levels may therefore influence serotonin synthesis. Natural or experimental
changes in tryptophan levels can be used to prospectively investigate the role of a decreased mono-aminergic functioning on mood and motivation (31), (41).

**Stress and HPA-axis functioning**

One of the most robust epidemiological findings regarding the onset of depression is that an episode of depression is often precipitated by a stressful life-event (42-44). This observation induced a lot of research into the consequences of stress on brain functioning, behavior, and the pathogenesis of depression. Stress may be described as any environmental challenge, either internally or externally, that disturbs homeostasis (44). The term stress can be used in two ways; either to describe events or circumstances that are perceived adversely (stressor) or the state induced by such events or circumstances (the stress response) (45).

The stress response consists of two phases. During the acute phase the sympathetic nervous system is activated, resulting in the central and peripheral release of epinephrine and norepinephrine into the circulation. Both hormones stimulate $\alpha$ and $\beta$ adrenergic receptors in the vessel wall and heart muscle, thereby causing an increase in heart rate and blood pressure. During the second, delayed phase, the HPA-axis is activated, resulting in the adrenal release of cortisol into the blood stream. Cortisol facilitates the maintenance of the stress response by mobilizing energy from energy stores; it prepares the body for injury by inhibiting the immune system and stimulating blood clotting and it facilitates coping with (future) stressors by influencing memory and learning processes.

As dysfunctioning of the HPA-axis is considered to be one of the main causes of depression, this system will be considered in more detail, and evidence for its role in depression will be summarized.

**The HPA-axis**

Stimuli that are perceived or labeled as stressful by the cerebral cortex lead to stimulation of neurons in the paraventricular nucleus of the hypothalamus (PVN) to produce corticotropin releasing hormone (CRH), which is released into portal blood stream of the pituitary. CRH stimulates the pituitary gland to release adrenocorticotropin releasing hormone (ACTH), which in turn stimulates the adrenals to synthesize cortisol and release it into the systemic circulation. This whole process takes about 20-30 minutes. The overshoot of HPA-axis activity is limited by negative feedback mechanisms, i.e. cortisol inhibits the production of both CRH and ACTH (figure 3). In
non-stressful situations, cortisol secretion follows a circadian pattern, peaking early in the morning and steadily declining during the day.

**The HPA-axis and depression**

Research into the HPA-axis functioning of depressed patients designated that a substantial group of patients have increased HPA-axis activity (46-48), as indicated by increased levels of cortisol in urine (49-51), and cerebrospinal fluid (52), as well as enlarged adrenal-, (53) and pituitary glands (54). However, atypical depression (55) has been associated with diminished HPA-axis activity (56). Measures of HPA-axis functioning also show disturbances. The circadian rhythm of cortisol is flattened in depressed patients (57-59), and the stress response is increased, mainly due to a slow recovery (47). Using dexamethasone or dexamethasone + CRH administration, to evaluate the sensitivity for feedback of the pituitary gland and hypothalamus respectively, it has been shown that the normal reaction of cortisol suppression is absent in almost half of the patients (50, 60, 61), which can be normalized following SSRI treatment (62).

The etiological relation between these HPA-axis disturbances and depression is still unclear. On the one hand it is suggested that an excess of cortisol induces depression, as indicated by the high incidence of depression in patients treated with corticosteroids (63), or with Cushing syndrome (64), and the suggested neurotoxic effects of cortisol (65). On the other hand it is hypothesized that severe stress might dysregulate the delicately balanced feedback mechanisms of the HPA-axis, thereby increasing the neural vulnerability to stress (66). Finally, these disturbances might be trait markers for a vulnerability for depression, as healthy volunteers with depressed family members also have a disturbed HPA-axis stress response (67).

Thus, HPA-axis dysfunctions may cause depression or might be trait- or state markers of depression. As (the direction of) the causality of the associations between HPA-axis functioning and depression is still unclear, this subject might benefit from prospective psychiatric research in hypercortisolemic patients.
**Genetic aspects**

The heritability of depression, as estimated in twin studies, is 37% (68), but the genetic features of depression are complex, and poorly understood. Genetic association studies have so far identified six ‘modifying genes’, but odds ratios are small (0.51-2.06) (69). Other approaches link genetics to characteristics related to depression e.g. personality (70, 71), emotional processing (72), stress reactivity (73), and sensitivity to tryptophan depletion (34), but all with very mixed results.

The most successful genetic approach till now was introduced by Caspi et al. in 2003 (74), who reported that carriers of a functional polymorphism of the serotonin transporter, with a history of childhood maltreatment developed depression after several life events. This ‘gene x environment interaction’ has been replicated with several studies (75). However, this approach has some limitations, as the relation between the onset of depression and the preceding life event is not straightforward due to a great variety of included stressful events and a highly variable time between the event and the onset of depression. The ideal design to falsify this concept should therefore consist of a more or less “standardized life event”, followed by psychiatric assessments at fixed time intervals.

In this thesis the focus will be on polymorphisms in three candidate genes involved in mono-aminergic metabolism, all of which have been implicated in processes involved in the onset of depression, i.e. polymorphisms in the genes of the serotonin transporter (5-HTT), mono-amine-oxidase type A (MAOA) and catechol-O-methyl-transferase (COMT). Function of these 3 proteins is illustrated in figure 4.

The 5-HTT gene located at 17q11.1-q12, codes for a protein with a key role in the regulation of extracellular serotonin levels. A short allele (s) or a long allele with an A-to-G substitution (l) in the 5-HTT promoter region affect the expression and functionality of the 5-HTT (7, 8), the long (l) allele in this promoter region, corresponds for a normal functioning variant of the 5-HTT. The gene coding for MAOA, mapped to the short arm of the X chromosome, translates into an enzyme involved in the degradation of serotonin, dopamine and noradrenalin. The promoter region of this gene contains a variable-number-tandem-repeat polymorphism (VNTR’s), consisting of a 30-bp repeated sequence, that occurs as 2, 3, 3.5, 4 or 5 repeats (R). The 3.5R or 4R are transcribed 10 times more efficiently compared to the other variants i.e. the 2R ,3R, 5R (13). Finally, the COMT-gene, which is mapped on chromosome 22, codes for an enzyme involved in the deactivation of dopamine and noradrenalin. A G-to-A substitution in codon 158, translating into a valine (val) to methionine (met) substitution, has been shown to account for a fourfold decrease in enzyme activity (14).
The 5-HTT s and lC polymorphism are associated with depression (69), particularly in interaction with stressful life events (80, 81). The long (l) allele is found to modulate the depressive symptoms induced by tryptophan depletion (34). The MAOA low activity variants have been associated to depression, sleep disturbances, and neural processes involved in the onset or persistence of depression (88-92). Finally, the COMT low activity variants have been related to an altered stress-response and to processes involved in the onset of depression (82-87). In addition, the combination of both MAOA and COMT low activity variants is found to be associated to an altered stress response and suicidal behavior (73, 93).

Female gonadal hormones
The incidence of depression is twice as high in women (21.3%) as in men (12.7%) (94). This difference emerges during puberty, and disappears after the menopausal transition (95, 96). In addition the incidence of depression is found to be increased in periods characterized by changes in gonadal functioning, like the pre-menstrual period (97), pregnancy (98), and the peri-menopause (95, 99, 100). These findings suggest that the onset of depression is related to changes in levels of the female gonadal hormones estrogen (101) and progesterone (102).

More evidence is found in pre-clinical research. Estrogen receptors are found all over the brain (103, 104), and they have been found to influence functioning of the different monoamine systems (105, 106), neuronal plasticity related processes (107), and brain areas related to the pathophysiology of depression, like functioning of the HPA axis (108, 109) and hippocampus (110). Likewise, progestin receptors are found to exert
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effects on different brain areas (111, 112). However, the main influence of progesterone on the brain is due to the progesterone metabolite 3α-OH-5α-pregn-20-one (allopregnanolone) (113, 114). Allopregnanolone modulates the GABA\textsubscript{A} (\(\gamma\)-aminobutyric-acid-A) receptor thereby enhancing the inhibitory effects of the neurotransmitter GABA on the brain (115), thus influencing a wide range of brain functions (116, 117).

Despite the evidence supporting a role of these hormones in depression it remains unclear why only a subgroup of women develops depression, since all women are exposed to the cyclic changes of these hormones. It has therefore been proposed that some women have a specific vulnerability for these endocrine events (97, 118), but a “substrate” for this vulnerability has not been identified so far (119).

**Omega-3 and 6 fatty acids**
The long chain polyunsaturated fatty acids (LCP) docosahexaenoic acid (DHA, 22:6 omega-3) and arachidonic acid (AA, 20:4 omega-6) are important structural components of brain phospholipids, precursors of eicosanoids and modulators of gene expression and are known to influence neurodevelopment (120). DHA, but also eicosapentaenoic acid (EPA, 20:5\(\omega\)3) are mainly derived from fatty fish, while meat and eggs are the principal dietary sources of AA. The low fish consumption in most western countries likely causes low EPA and DHA levels in of their inhabitants (121). In epidemiological studies this condition has repeatedly been implicated in depression (122-124). Case-control studies investigating LCP status reported that patients suffering from depression have lower levels of omega-3 fatty acids and a concomitant higher omega-6 /omega-3 ratio (125). Most meta-analyses of clinical trials with omega-3 fatty acids for depression suggest that they might be effective, however, the effect sizes are small (126, 127). One meta-analyses found little support for anti-depressive effects of omega-3 fatty acids (128).

There are numerous pathways by which fatty acids may be linked to depression. First, DHA and AA are structural components of (neural) cell membranes. DHA and AA influence membrane fluidity, and thereby functioning of membrane bound enzymes, ion channels and receptors (129); Second, omega-3 fatty acids influence the Brain Derived Neurotrophic Factor (BDNF) concentration, thereby promoting cell survival and neuroplasticity. Finally poly-unsaturated fatty acids have immuno modulating properties and affect the production of pro-inflammatory cytokines and prostaglandins (130). Inflammatory processes have been related to depression, and increased levels of pro-inflammatory cytokines have been found in depressed patients (131).
Conclusive remarks and a proposal for studies in naturalistic life-events

The inconsistent results of biological explanations for depression might originate from the heterogeneous character of depression. In other words, it may be that “different types” of depression have been included in research, thus causing the inconsistency of the results. Research in depression might therefore benefit from inclusion of homogeneous cohorts. In addition it might be fruitful not to investigate the diagnosis depression as an entity, but to disentangle this concept and investigate more specific and better defined entities like the development or recovery of specific symptoms and the severity of symptoms.

Evidence has been documented for all these biological theories on depression but, as shown, most of the findings are inconsistent associations, and it remains unclear whether the alterations in the different systems are causally linked to depression or have to be considered as state markers for depression. The role of these systems in the pathogenesis of depression might be elucidated by prospective studies in naturally occurring disturbances of the HPA-axis, monoamine metabolism, gonadal functioning or altered fatty acids. Moreover, as stressful events seem to contribute to the onset of depression, involvement of a stressful situation in this period would increase the etiological validity of the investigation performed in this situation.

Here we propose pregnancy as a period that enables this kind of investigations, as pregnancy is characterized by a decrease in serotonin functioning, HPA-axis disturbances, large changes in levels of estrogen and progesterone and a decrease in omega-3 fatty acids. Moreover, pregnancy, but mainly parturition, can be considered as a major life event, whereas severely complicated pregnancies are major life events that are also stressful. Furthermore, pregnant women are quite a homogeneous group in terms of gender and age. Finally, prospective research in pregnancy is facilitated by the predictable course of pregnancy and the well-organized care for pregnant women.

In the following paragraphs this idea will be further elaborated; the functioning of the above mentioned systems during pregnancy will be described, and their relation with affective symptoms in this period will be summarized. To begin with, a summary will be given of the literature considering the incidence of affective disorders during pregnancy and postpartum.

Peripartum affective disorders: incidence, course and risk-factors

Two separate affective syndromes can be distinguished in the peripartum period: major depressive disorder, which can be labeled as postpartum onset in the DSM IV (4); and
postpartum blues: a transient, benign affective syndrome occurring in the first week postpartum and lasting about 1-2 days (132).

**Depression**

The incidence of depression in the peripartum period has been a subject of debate for the past years. Two systematic reviews regarding this subject have been published recently. Bennet et al. (133) estimated the prevalence of depression during pregnancy for the three trimester as 7.4%; 12.8% and 12.0%, respectively. Included were studies using both systematic interviews and questionnaires. Gavin et al. (134, 135) reviewed both pregnancy and the first postpartum year and excluded studies using questionnaires and studies performed in less-developed countries. They calculated the monthly prevalence for the whole period as 5.7%, which did not differ from the monthly prevalence of depression in women aged 15-54 in the USA. However, due to large confidence intervals, no definite conclusions could be drawn. Recently quite reliable incidence rates for psychiatric disorders during pregnancy have been calculated with data from a large epidemiological study, using standardized interviews (98). These data indicated that the 12 months prevalence of depression was somewhat increased in the postpartum period (9.3 % Standard Error %, 1.1) compared to non-pregnant women (8.1%, SE 0.9). However, these estimates might not apply for non-western countries (136). The prevalence of suicidality is decreased in the peripartum period (137).

Most women who were depressed during pregnancy were also depressed in the months preceding (138) and following pregnancy (139-141). Risk factors for postpartum depression have been summarized by Robertson et al. (139) and indicate that strong risk factors are: a past history of psychiatric illness, life events, and lack of social support; moderate risk factors: neuroticism and (poor) quality of marital relationship, and as less important risk factors: obstetric factors and socioeconomic status. Bloch et al. (142) further indicated that putatively hormone-related phenomena, such as Premenstrual Dysphoric Disorder are related to the occurrence of postpartum mood disorders.

Peripartum depression has pertinent consequences for the development of the child, e.g. decreased birth weight (143-145), less favorable maternal attachment (146), decreased (intellectual) development in the first year (147, 148), behavior problems at 4-5 years (149, 150), aggressive behavior and ADHD (151), and increased risk of depression at 11 and 17 yrs (152, 153).
Depression questionnaires
The uncomplicated pregnancy is accompanied by somatic complaints like fatigue and sleeping problems, which are also symptoms of depression. Therefore these symptoms lose their specificity for depression. To circumvent this problem, a special questionnaire for peripartum depression excluding those somatic complaints has been developed by Cox et al.; the Edinburgh Postnatal Depression scale (EPDS) (154). The EPDS is the most frequently used scale worldwide, and has moderate psychometric properties. The EPDS is accepted for pregnant women (155), and validated in countries all over the world (156) including the Netherlands (157). A score of 13 or more indicates that women are at risk of having a depression (158), but the EPDS can also be used to evaluate the course of the disease and the effects of intervention (159). The EPDS, however, is also criticized since it ignores psychomotor retardation (160) and also measures anxiety (161). The underlying symptom structure is multidimensional (162) and Rasch analyses indicate that an 8 item scale would be more robust (163), thus limiting the qualifications of the EPDS for routine screening (164).

Blues
Symptoms of postpartum blues consist of weeping, mostly without feeling depressed (165), emotional lability with mood changes all over the day (166), anxiety, confusion, headache and self reported cognitive impairment (167, 168) but also elation (169). There is agreement in literature that the blues starts the first few days after delivery and stops by 7-10 days postpartum (132). The highest symptom scores have been found on days 3 and 4 postpartum (132, 170). The reported prevalence rates range from 15 to 84% (132, 171), but most studies found prevalence rates of about 50% (168). Postpartum Blues has been described among different cultures (132), suggesting that it is a cross-cultural phenomenon.

Risk factors for blues have been summarized by Henshaw (132). The most convincing relationships according to his analyses were dysphoria during pregnancy, past history of depression, neuroticism and pre-menstrual dysphoria. Blues is considered to be a risk factor for postpartum depression (132, 142, 172).

The only specific and validated scale for postpartum blues is the Postpartum Blues Scale, which was developed by Kennerley and Gath (170, 173, 174).
Physiological aspects of pregnancy and their relation to depression

Serotonergic metabolism, pregnancy and depression
During the peripartum, the synthesis of both peripheral and cerebral serotonin is limited due to the decreased availability of tryptophan. This is caused by the increased catabolism of tryptophan by placental IDO (175-178), resulting in a 30% decrease in plasma tryptophan levels (179, 180, 175, 176, 181). Following delivery, tryptophan availability is limited due to IDO activity in the activated immune system and by increased liver metabolism by the tryptophan pyrrolase (182-184). During pregnancy, serotonergic activity is also modulated by estrogen, progesterone and some of their metabolites (185) resulting in a sudden change in the serotonergic neurotransmission postpartum. Taken together, pregnancy and the early postpartum period are characterized by a sudden change, mostly a reduction, of serotonergic activity. And, as clarified above, reduced activity of the serotonin system has been associated with the onset of depression.

Changes in tryptophan concentration (40, 186-188), tryptophan catabolism (184, 189) and platelet 5-HT content (190) have all been associated with postpartum blues. Moreover, postpartum depression has been associated with impaired activity of the platelet serotonin transporter (SERT) as reflected by a decreased dissociation constant of both imipramine and paroxetine (191, 192).

HPA-axis functioning, pregnancy and depression
During the course of pregnancy, the placenta produces CRH. Accordingly plasma CRH levels increase 30 fold and ACTH levels 5 fold (119, 193). Consequently, cortisol levels increase to the upper limit of the reference values. Part of this is compensated by a 2-fold increase in Corticotropin Binding Globulin (119). The circadian rhythm of cortisol is flattened (194). The dexamethasone suppression test (DST) does not induce the normal decrease of cortisol levels (195, 196), and the HPA-axis stress-response is diminished during pregnancy and postpartum (197). After delivery of the placenta, the CRH and cortisol levels return to normal values in three days. However, the disturbances of the DST continue for some weeks (198, 199), and are fully recovered only after six weeks. The persistent puerperal HPA-axis dysfunctions have been compared with the “postcure Cushing syndrome” (200). These HPA-axis disturbances are comparable to those associated with depression, i.e. hypercortisolism, a flattened circadian rhythm, a disturbed stress-response and the inability of dexamethasone to suppress cortisol levels.

Some studies report a relation between these HPA-axis disturbances and postpartum blues and depression. Women with blues have a significantly diminished cortisol response in the Dex/CRH test (201). Beside that low levels of saliva cortisol in the
evening in the first postpartum week have been correlated to postpartum depression (202). Other studies reported a relation between high plasma or saliva cortisol levels and blues (203-205). However, most studies failed to find a relation as the course of saliva or plasma cortisol levels did not correlate with postpartum blues (206-210), nor did the reaction on the DST correlate with blues (211).

**Estrogen, progesterone, pregnancy and depression**

During pregnancy estrogen levels increase to a 50 fold over maximum menstrual cycle levels by the time of the third trimester (212) and decreases to early follicular levels by day 1-3 postpartum (119). Progesterone levels increase to a 10 fold of maximum menstrual cycle levels (212), and follicular levels are reached by day 3 to 10 postpartum (213).

These peripartum changes in estrogen (166, 208, 209, 214, 215) and progesterone (210, 214, 216) or allopregnanolone (217) have been associated to blues and depression, but there is a great variety in direction of the correlations. Moreover, some studies report negative findings on this relation (202, 218, 219). As most studies are very small, no definite conclusions can be drawn on this relation. Trials investigating the effects of estrogen and progesterone treatment postpartum indicate that synthetic progestins can induce depressive symptoms, and that estrogen might be of modest preventive value. However, results are, according to the Cochrane review on this subject, inconclusive (220).

The strongest evidence for the role of estrogen and progesterone in the onset of postpartum affective symptoms is found in an experiment of Bloch and co-workers who reported that withdrawal of an eight week treatment of estrogen and progesterone induced depressive symptoms and disturbances of the HPA-axis in women with a history of postpartum depression (221, 222).

**Omega-3 and omega-6 fatty acids, pregnancy and depression**

The maternal LCP status declines during pregnancy and lactation, partly due to high fetal LCP needs (223). This condition has been related to the onset of peripartum depression. An epidemiological study showed that the highest incidence of postpartum depression occurs in countries that are characterized by the lowest fish consumption and breast milk DHA contents (224). Some observational studies showed that low DHA status predicted the occurrence of postpartum depression a few weeks later (225, 226). The relation between peripartum depression and LCP status is furthermore suggested in intervention studies. Treatment of peripartum depression with LCP omega-3 seemed effective in a small open label trial (227) and a randomized, placebo controlled clinical trial (RCT) (228), although other studies reported negative findings (229, 230).
Limitations of psychiatric investigations during pregnancy

Although the similarities between pregnancy and the suggested pathophysiology of depression are striking, the physiology of pregnancy is far more complex than the changes in the systems described here and consists of adaptations in functioning of virtually every physiological system in the body. Some adaptations that are most relevant for depression are: endocrine factors as changes in thyroid function (231, 232), prolactin (233) and vasopressin (234) levels (119); changes in functioning of the immune system (235, 236); adaptation of the circulation (237) and composition of blood (238), pregnancy related diabetes (239) sleep disturbances (240, 241), etc. Results of investigations of psychiatric functioning in this period should therefore be interpreted within the complex physiology of pregnancy.

Aim and outline the thesis

Depression has been associated with dysfunction of several mono-amine and endocrine systems, in interaction with stressful life events. However, it remains to be clarified whether the described physiological disturbances are causally related to depression or have to be considered as state or trait-markers of depression. The genetics of depression is complex, and has to be understood in interaction with stressful events. The physiology of pregnancy in some aspects, resembles the proposed pathophysiology of depression and can be considered as a major life event, as is shown above, and therefore offers the opportunity to prospectively investigate the relation between dysfunction in these systems, stressful events and the development of depression.

The aim of this thesis is to elucidate the role of the mono-amine system, HPA-axis and female gonadal hormones and omega-3 and omega-6 fatty acids, in interaction with genetic factors and stress in the development and persistence of depressive symptoms prospectively, i.e. during pregnancy and postpartum. With this approach the relation between functioning of the above mentioned systems and depression might be clarified.

In chapter 2, the behavioral effects of estrogen and progesterone withdrawal are investigated in an animal model. Subgroups of women seem to be vulnerable to the fast decrease in estrogen and progesterone following delivery; a difference in the capacity to adapt to the postpartum hormonal changes might underlie this vulnerability. This hypothesis is investigated by comparing the behavioral consequences of a rapid and a gradual decline of estrogen and progesterone.

In chapter 3, the focus is on the serotonin system. It has been suggested that peripartum depressive symptoms are caused by pregnancy related changes in monoamine
functioning. It was therefore investigated whether the onset of postpartum blues was related to changes in plasma parameters of serotonin and norepinephrine metabolism. For this reason blood was collected from 26 healthy pregnant women at the end of pregnancy and 5 days and 6 weeks postpartum, and a blues questionnaire was administered at day 5 postpartum.

The effects of the pregnancy related decrease in omega-3 fatty acids on mood is evaluated in chapter 4, which describes the results of a randomized controlled trial comparing supplementation of DHA and DHA + AA during pregnancy and postpartum to placebo.

The sample included in the above-mentioned trial is used to evaluate some genetic aspects of peripartum depression as well. In chapter 5, the influence of polymorphisms in MAOA, 5-HTT and COMT genes on the course of depressive symptoms during pregnancy and postpartum is investigated. In chapter 6, subgroups of women who develop depression in the peripartum are identified using a latent class analysis. These subgroups are associated with polymorphisms in the above-mentioned genes.

In the last two chapters, the effect of severe life events on the development of depression is evaluated in a group of pregnant women suffering from severe pregnancy complications, i.e. the HELLP syndrome (Hemolysis Elevated Liver enzymes and Low Platelets), preeclampsia and preterm pre labour rupture of the membranes (PPROM).

The incidence and risk factors for depression and post traumatic stress disorder following these disorders are described in chapter 7. In chapter 8, it is investigated whether the development of depression and post traumatic stress disorder in this sample is modified by polymorphisms in the serotonin transporter gene.

The results are summarized and discussed in chapter 9, which will conclude with some implications of this research for current ideas about the etiology of depression and with some ideas about future research in this field.
Chapter 1

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