The Stress Hypothesis

- Implications for the induction of diabetes-related autoimmunity in children?

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"The study of children
is too important to be left in the hands
of a single discipline"

(Dr. Ross Parke, 1991)

ABIS

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Background

Second to Finland, Sweden has the world's highest incidence of type 1 diabetes. Experiences of serious life events have retrospectively been shown to constitute a risk factor for the development of this disease, probably via the biological stress response. Parenting stress and maternal attachment insecurity are other important sources of stress in early childhood.

Psychological stress increases the need for insulin and may induce insulin resistance, which might add extra pressure on the insulin-producing beta cells in the pancreas (beta-cell stress).

The aim of the current thesis was to propose and start investigating a stress hypothesis – namely that psychological stress may induce insulin resistance leading to beta-cell stress, which could trigger an autoimmune reaction towards beta-cells in genetically predisposed children. When all the beta cells have been destroyed, insulin can no longer be produced in the body and type 1 diabetes becomes manifest.

Methods

Families from the prospective population-based ABIS-project, which follows approximately 17 000 children, participated in the empirical studies of the current thesis. The mothers completed questionnaires, including various measures of psychological stress (e.g. parenting stress and experiences of serious life events) and socio-demographic background, at the birth of the child and when the child was 1 as well as 2.5 years of age. Maternal attachment insecurity was assessed with the Adult Attachment Interview. Blood samples drawn from the children at 1 and 2.5 years of age were analyzed for type 1 diabetes-related autoantibodies towards Tyrosine phosphatase (IA-2) and Glutamic Acid Decarboxylase (GAD).

Findings and Conclusions

Parenting stress and experiences of serious life events like divorce and maternal exposure to violence were associated with the induction of diabetes-related autoimmunity in early childhood, possibly via insulin resistance and beta-cell stress. The risk of developing diabetes-related autoimmunity after parental divorce or mothers' exposure to violence was about threefold. None of the results were explained by any of the potential confounding factors analyzed. These results support and strengthen the stress hypothesis, which warrants further investigation.

Mothers' attachment insecurity was not associated with the induction of diabetes-related autoimmunity in their infants. However, this lack of association was perhaps due to methodological constraints.

The vast majority of the parents were calmed or unaffected concerning their participation in the ABIS-project, suggesting that large-scale medical screening-projects in the general population are not in themselves a cause for worry and can be performed without causing increased anxiety.

Abbreviations

AAI	The Adult Attachment Interview
ABIS	All Babies In southeast Sweden
IA-2A	Autoantibodies towards Tyrosine phosphatase (diabetes-related)
IA-2	Tyrosine phosphatase
IAA	Insulin autoantibodies (diabetes-related)
ICA	Islet cell autoantibodies (diabetes-related)
IWM	Internal working model
CI	95% Confidence interval
GADA	Autoantibodies towards Glutamic Acid Decarboxylase (diabetes-related)
GAD	Glutamic Acid Decarboxylase
IWM	Internal working model (i.e. mental representation or mental model)
OR	Odds Ratio
NICU	Neonatal intensive care unit
RSQ	Relationship Scales Questionnaire
SES	Socio-economic status
SPSQ	The Swedish Parenthood Stress Questionnaire
T1D	Type 1 diabetes
TT	Tentanus Toxoid

Original publications

The present thesis is based on the following studies, conducted within the ABIS-project, which will be referred to in the text by their Roman numerals:

I. Ludvigsson, J., Ludvigsson, M., & Sepa, A. (2001). Screening for prediabetes in the general child population: maternal attitude to participation. *Pediatric Diabetes*, *2,* 170-174.

II. Sepa, A., Frodi, A., & Ludvigsson, J. (2002). Could parental stress and lack of support/confidence function as mediating mechanisms between certain environmental factors and the development of autoimmunity in children? - A study within ABIS. *Annals of the New York Academy of Science*, *958,* 431-435.

III. Sepa, A., Frodi, A., & Ludvigsson, J. (2004). Psychosocial correlates of parenting stress, lack of support and lack of confidence - A study of all babies in Southeast Sweden (ABIS). *Scandinavian Journal of Psychology, 45,* 167-177

IV. Sepa, A., Wahlberg, J., Vaarala, O., Frodi, A., & Ludvigsson, J. Psychological stress may induce diabetes-related autoimmunity in children. (manuscript to be submitted within shortly)

V. Sepa, A., Frodi, A., & Ludvigsson, J. Mothers' attachment insecurity and diabetes-related autoantibodies in their infants. (manuscript in preparation)

VI. Sepa, A., Frodi, A., & Ludvigsson, J. Experiences of serious life events and diabetes-related autoimmunity in children. (manuscript in preparation)

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INTRODUCTION

First I will present a little about type 1 diabetes and diabetes-related auto-immunity, since this is important for the stress hypothesis proposed, although medicine is not the main topic of the present thesis. Potential etiological factors of type 1 diabetes will be outline in somewhat more detail. After that, the issue of psychological stress and especially sources of stress during early childhood will be dealt with, and the introduction will end with the aims and research questions of the current thesis.

Type 1 diabetes and autoimmunity

Type 1 diabetes (T1D) is one of the most common chronic diseases among children in Sweden and close to 600 children below 15 years of age are newly diagnosed every year. T1D is a severe and life-long disease demanding con-siderable self-discipline concerning daily self-managed treatment, which is a burden especially for children and adolescents.

The normal function of the immune system is to protect the body, but sometimes and for some reasons the immune system can start to attack and destroy its own body tissues. This is called an autoimmune reaction or an autoimmune disease. According to present knowledge, Type 1 diabetes becomes manifest when the immune system has destroyed all beta cells in the pancreas so that insulin no longer can be produced in the body. Absolute lack of insulin is fatal, since the insulin is necessary to supply the cells with energy (glucose) for survival.

Type 1 diabetes is defined by insulin-dependency and autoimmunity to insulin-producing beta-cells.

Prediction

Clinical onset of T1D is often preceded by the presence of autoantibodies (e.g. IA-2A, GADA, ICA, and IAA) circulating in the blood and their detection is widely used to identify subjects at increased risk of developing T1D. Multiple autoantibody positivity is believed to best predict progression of T1D (Bingley, 1997), but some studies have shown that IA-2A alone is also a good predictor (Decochez et al., 2002; Savola et al., 1998). One of these studies found that IA-2A alone, even if only detected once during a 5 year pre-diagnosis period, was

the best predictor representing a 60% risk of progression to diabetes within 5 years (Decochez et al., 2002).

Epidemiology

There has been a rapid increase in the incidence of T1D in many European countries in the last few decades with a higher rate of increase among children under 5 years of age (Green, Patterson, on behalf of the EURODIAB TRIGR Study Group, 2001; EURODIAB ACE Study Group, 2000). The incidence in Europe during 1989-1998 ranged from 3.6 cases per 100 000 per year in Macedonia to 43.9 cases per 100 000 per year in Finland (Green et al., 2001).

Second only to Finland and Sardinia in Italy, Sweden has the world's highest incidence of T1D (Green et al., 2001). The mean incidence in Sweden during 1995-1998 was 32 per 100 000 children per year (Pundziute-Lycka et al., 2002), and it has increased even more in the last few years (Swedish National Register of Childhood Diabetes). Some parts of Sweden (in the province of Östergötland) have had an incidence as high as 52/100 000 children per year during the last 25 years (Samuelsson, 2003, personal communication). However, according to one study, the T1D incidence in the age group 0-34 years in Sweden has not increased during 1983-1998, but there has been a shift to a younger age at diagnosis, which seems to be the explanation for the increasing incidence of childhood T1D (Pundziute-Lycka et al., 2002).

Etiology

T1D is thought to develop in **genetically** predisposed individuals as a result of a progressive autoimmune destruction of the insulin producing beta cells in the pancreas. Nevertheless, the concordance rate of T1D in monozygotic twin pairs is relatively low, approximately 35-50% (Hawkes, 1997), and only about 10% of all newly diagnosed have a first degree relative with T1D at the time of diagnosis (Dahlquist & Mustonen, 2000). It is still not known why the immune system starts to attack its own healthy body tissues, but these facts as well as the rapidly increasing incidence, point to the importance of environmental factors in the development of T1D.

A number of environmental factors have been proposed as trigger mechanisms of this autoimmune beta-cell destruction. Different kinds of **viral infections**, both pre- and post natal, are usually listed first among the environmental risk factors in the etiology of Type 1 diabetes. Cases of diabetes onset after Chickenpox and Rubella have been described more than 100 years ago. More

recently enteroviruses have been suggested to play a role in the development of diabetes (Hyöty & Taylor, 2002).

The first study suggesting that **dietary components** may markedly affect the expression of diabetes in rats was published in 1983 (Scott & Trick, 1983), especially wheat and soy protein gave a higher incidence of diabetes in rats, and shortly thereafter cow milk proteins were suggested as a trigger mechanism (Elliott & Martin, 1984). Today, introduction of cow's milk and gluten are studied as potential risk factors (Knip, 2003; Åkerblom & Knip, 1998).

In retrospective studies, **increased maternal**, as well as **paternal age** have been linked to type 1 diabetes in the child (Bingley et al., 2000; McKinney et al., 1999; McKinney et al., 1997). Bingley et al. (2000), for example, reported an increased risk of 25% (CI: 17-34%) for each five year band of maternal age, so that a maternal age of 45 years or more at delivery was associated with a relative risk of 3.1 (CI: 2.1-4.7) compared with a maternal age of less than 20 years. Concerning the age of the father each additional five year span was associated with a 9% (CI: 3-16%) increase of the risk. Whether the increased risk associated with age depends on somatic changes or on psychological mechanisms remains to be shown.

In retrospective studies **caesarean section** and need for **neonatal intensive care** have been found to constitute risk factors for the development of Type 1 diabetes (McKinney et al., 1997; McKinney et al., 1999). What these associations stand for is not known, but one could speculate that the association could be, at least partly, explained by low birth weight and excessive compensatory weight gain (Johansson, Samuelsson, & Ludvigsson, 1994).

Kolb and Elliot (1994) have suggested that the **hygiene hypothesis**, originally introduced within the field of allergy research, could be linked to T1D too. Accordingly, the increasing incidence of Type 1 diabetes seen in the industrialized world may be due to better hygiene resulting in reduced exposure to microbial antigens early in life and a reduced need for a strong immune defense. This hypothesis is partly supported by the fact that the incidence is reduced in rodents exposed to high doses of microbial antigens early in life, but no direct evidence supporting an association in humans has yet been presented.

The **accelerator hypothesis**, proposed by Wilkin (2001), argues that all types of diabetes (type I and type II) are the same disease only differentiated by the rate of beta-cell loss and the accelerators responsible for this beta-cell loss. Three accelerators (processes) are identified which variably accelerate the loss of beta cells. The first accelerator is an inborn high rate of beta-cell apoptosis (natural

programmed cell death), which is necessary but not sufficient for the development of diabetes. The second accelerator is insulin resistance. According to the accelerator hypothesis, insulin resistance is caused by excessive weight gain and physical inactivity, which further increase the rate of beta-cell apoptosis. Finally, beta-cell autoimmunity, the third accelerator, is thought to develop in a small, genetically defined, subset of individuals with both intrinsic lesion (accelerator 1) and insulin resistance (accelerator 2). Wilkin regarded insulin resistance as the primary accelerator and the common basis for type I and type II diabetes (Wilkin, 2002). Insulin resistance could also be expected to increase the beta-cell stress and to intensify an autoimmune response in those who are genetically predisposed (Wilkin, 2001).

However, weight gain and physical inactivity are not the only phenomena causing insulin resistance. The **beta-cell stress hypothesis**, proposed by professor Johnny Ludvigsson (personal communication), suggests that any phenomenon that induces insulin resistance and thereby adds extra pressure on the beta cells should be regarded as a risk factor for diabetes. Psychological stress, for example, is a factor well known to decrease insulin sensitivity and increase insulin resistance, and, therefore, psychological stress might play an important role in the development of diabetes.

Psychological stress as a risk factor for type 1 diabetes

The notion that psychological stress could cause diabetes is not new. Very early on, prolonged sorrow (Willis, 1679) and stress (Osler, 1892; Maudsley, 1899) were suggested as etiological factors in diabetes. In 1935 anxiety and depression were suggested to be characteristics of the "diabetic personality" (Menning, 1935) and some further support concerning the etiological role of stress in the development of diabetes based on case reports was published before 1980 (e.g. Hinkle et al., 1951; Danowski, 1965; Stein & Charles, 1975). For a critical review of older references, see Johnson (1980).

Many diabetic patients also believe that their diabetes has been caused by stress or a serious life event, whether this is true or due to a wish of finding external causing mechanisms is a question that research must still answer.

There are some more modern studies that have addressed the issue of psychological stress as a contributor to diabetes. Table 1 is a summary of the 8 studies on humans performed to address this issue during the last 20 years (known to the author). All of these studies found an association between life events (of different kinds) and the development/onset of type 1 diabetes. However, they were all retrospective case-control studies, so the question of causality still remains to

be addressed. Three of these studies have addressed the onset of T1D in childhood. One of them found that stressful life events, related to actual or threatened losses within the family, occurring during the year prior to diagnosis were associated with the onset of childhood diabetes in the vulnerable age-group of 5-9 years (Hägglöf et al., 1991). The other two studies, based on the same population, found that negative life events during the first two years of life represented an increased risk for later onset of T1D (Hägglöf et al., 1994; Thernlund et al., 1995).

A couple of review articles have stated that there is insufficient evidence to support the position that psychological factors directly affect the onset of diabetes mellitus (Wales, 1995; Beardsley & Goldstein, 1993). However, Wales found it likely that stress could produce a drop in glycaemia in non-symptomatic patients, which in turn would make the diabetic symptoms and the diagnosis apparent (1995). Beardsley and Goldstein noted that recent laboratory studies had suggested an association between stress and changes in glucose regulation and that temperament and coping strategies could influence glycaemic control in diabetic children and adolescents (1993).

Table 1: *Summary of studies performed, to investigate whether psychological stress causes type 1 diabetes, during the last 20 years.*

Reference	Design	N	Age Range (mean ± SD)	Kind of stress measured	Result	Conclusion
Robinson & Fuller (1985)	Retrospective case-control study. Part of the Barts-Windsor-Middlesex Prospective family study	13 cases (newly diagnosed) 13 siblings (aged within 7 yrs of diabetic proband) 13 neighbors (sex and age matched with cases)	17-34 (22±?) 6 cases, 6 siblings, and 6 neighbors were 17-20 and the reminder were >21 yr of age.	Life Events and Difficulties Schedule (individual interviews). Life events and difficulties occurred during a 3 yr prior to diagnosis) "Severe life event": an event with marked/moderate threat lasting for 10 days or more. "Severe long-term difficulty": on-going problem lasting >4 weeks, rated <3 on 7-point scale ("marked" to "none").	Cases had a higher frequency of one or more severe life events 6 mo and 2.5yr prior to diagnosis compared to siblings and neighbors. Two or more severe life events prior to diagnosis: 54% in cases, 8% in siblings, and 8% in neighbors. Cases had a higher percentage and a higher mean number of severe difficulties than siblings and neighbors.	"Stressful life events and difficulties may be triggering factors involved in the aetiology of insulin-dependent diabetes." (p. 583)
Kisch (1985)	Retrospective case-control study.	66 T1D patients Duration of T1D: <3yr: 19 3-10yr: 15 >10yr: 32 62 controls (newly operated upon for acute surgical problems; age and sex matched)	<20yr: 2 21-40yr: 15 41-60yr: 34 >61yr: 15	Questionnaire: Events – physical or psychological – preceding the onset of their diabetes.	Febrile disease: 2 cases (2 controls) Accidents: 7 (2) Pregnancy: 2 (0) Problems in family or at work: 16 (5) Other: 22 (10) No event: 17 (43) Interval: event – T1D diagnosis (cases): within days: 10 within weeks: 11 within moths: 24 don't remember: 4 Sign difference between number of events in cases and controls.	"… although our data do not purport to prove an etiological link between stress and the onset of type 1 diabetes, the fact that 74% of our patients reported a stressful life situation preceding their disease is thought provoking and should encourage further investigation". (p. 358)

Study	Design	Sample	Age / Duration	Measures	Results	Conclusions
Vialettes et al. (1989)	Retrospective case-control study	32 cases (newly diagnosed) 53 controls (age matched)	Cases: 15-40 (25.7±?) Controls: 19-40 (28.9±?)	Life events (37 items dealing with health, family, and various other aspects of affective, professional, and social life). Subjects judged each event as stressful or non-stressful.	Cases reported a sign lower incidence of stressful and non-stressful life events during the last 20 yr before diagnosis. Cases reported a significantly higher incidence of stressful life events the year prior to diagnosis. 50% of cases (18.8% cont) had experienced 1 or more stressful life event the year prior to diagnosis.	"… in at least some patients stress may be a mediating factor." (p. 50) "…these findings do not attest to a temporal relation between stress and type 1 diabetes in at least 1 out of 2 patients, they do not establish a causative connection." (p. 45)
Robinson et al. (1989)	Retrospective case-control study. Parents and all siblings were screened for ICA every 4-6 month during 5 yrs. Retrospective assessment of life events (1 yr after diagnosis of the 2nd family member). Part of the Barts-Windsor-Middlesex Prospective family study	12 families with 1 T1D patient each. In 6 of the families ICA-positivity was noted in a 2nd member (case) and in 4 of the families did this person get T1D during the study. In 6 of the families, no further ICA-positivity was found. (control)	1st T1D: case: 4±2 control: 13±4 Duration: case: 15±5 control: 8±4 2nd T1D: case: 27±15 Duration: case: 3±3	Life Events and Difficulties Schedule (individual interviews with all family members). Life events and difficulties occurring to the family during 5 yrs prior to the 2nd T1D diagnosis were assessed. "Severe life event": an event with marked/moderate threat lasting for 10 days or more. "Severe long-term difficulty": on-going problem lasting >4 weeks, rated <3 on 7-point scale ("marked" to "none"). Level of social support from family, relatives, neighbors, colleagues, and friends.	Student's paired t-tests. Case families had higher scores for severe life events and severe long-term difficulties than control families. Each of the four who developed T1D during the study had experienced at least one or more severe life event within 5 yrs prior to diagnosis. Social support (no of current visual contacts) was better in control families than in case families. No difference in non-visual contacts.	"… global family stress possibly in conjunction with a reduced number of social contacts may act as a trigger for the development of diabetes in a second family member and that social support may act as a buffer to stress and disease onset." (p. 53)

Hägglöf et al. (1991)	Retrospective case-control study. Part of a nationwide Swedish case-referent study, where all newly diagnosed during 1 year were invited to participate.	338 cases (Newly diagnosed) 528 controls (Matched for age, sex, and geographical distribution)	0-14 (?±?)	Life events (45 items, including 15 items assessing losses or threatened losses in the family; Based on Coddington's scale)	Total number of life events the year prior to diagnosis did not differ, but life events experienced by cases (aged 5-9 yrs) were more severe (i.e. actual or threatened loss within the family) compared to controls.	"... stressful life events, related to actual or threatened losses within the family, occurring in the vulnerable age-group of 5-9 years, are associated with the onset of childhood diabetes. Such stressful events may in fact be a risk factor for the disease." (p. 579)
Hägglöf et al. (1994)	Retrospective case-control study, mainly addressing the issue of coping with the T1D diagnosis.	67 cases (newly diagnosed) 61 controls Geographically, sex, and age matched.	1-14 (9±?)	48 different life events (based on Coddington's scale) from birth to the onset of T1D were recorded 2 month after diagnosis. Child behavior Family function Social network	Neither the total number of life events nor any life event during last year prior to diagnosis differed between cases and controls. Cases had experienced sign more negative life events during first two yrs of life and sign more global stress during whole life compared to controls. Child behavior: cases had significantly more acting-out symptoms than controls. Family function: cases had a slight overrepresentation (p=.05) of the chaotic but not disengaged pattern concerning. Social network: no difference.	No conclusion concerning life events and onset of T1D.

Thernlund et al. (1995)	Retrospective case-control study. Part of the study performed by Hägglöf et al., 1994, described above.	67 cases (newly diagnosed) 61 controls Matched: age, sex, and socio-economic status (same class or daycare)	0-14 (8.3±4.0)	27 negative and 13 other life events (based on Coddington's scale), occurring during hole life span Child behavior Family function Parental social support	Negative life events during the first two years of life, life event with difficult adaptation, and more chaotic family function were more common in the case group.	"Stress early in life may increase the risk for T1D, presumably by affecting the autoimmune process." (p. 1323)
Littorin et al. (2001)	Retrospective case-control study.	349 cases (newly diagnosed) 979 controls Matched: age and sex.	15-34 (23 ± 6)	26 positive and negative life events (modification of Sarason's Life-Event Survey). Life Event Scale (degree of stress on analogue scale from extremely positive to extremely negative).	Serious illness/injury or hospitalized (> 1 week) within the yr prior to diagnosis represented an increased risk for T1D. Results concerning psychosocial life events were not as clear.	"... does not directly support the concept that psychosocial stressful life events are involved in the development of autoimmune type 1 diabetes in young adults." (p.1033)

Summary of the issue of Type 1 diabetes

The current review pointed out some known risk-factors for type-1 diabetes mellitus (of a biological nature) that might influence the occurrence of beta-cell autoantibodies. Therefore, in the analyses (in paper IV and VI) concerning psychological risk factors for beta-cell autoimmunity the following potential confounding factors were controlled for: diabetes heredity, child infections, exclusive breastfeeding, increased parental age, caesarean section, and need for neonatal intensive care. Further details concerning their assessments will be found in the method section.

A number of studies have suggested that psychological stress may play a role in the onset of type 1 diabetes. Unfortunately, these studies have been either case reports or retrospective case-control studies, making causal conclusions impossible. However, since psychological stress has been identified as a potential risk factor in most of the studies performed so far, the need for prospective longitudinal cohort studies to further illuminate possible causality has been urgent.

An additional problem with the retrospective studies is the fact that having a child who is diagnosed with type 1 diabetes is in itself a very stressful life event for the whole family. Therefore, it would be better to investigate the association between psychological stress and diabetes-related autoimmunity, since the child does not have any symptoms and the parents are unaware of the child's auto-antibody status, and thus not biased by any such knowledge.

Another constraint of the studies performed so far is the incomplete range of sources of psychological stress that was investigated. The focus has been on psychological stress caused by severe life events, which of course is an important source of stress. However, the autoimmunity thought to precede manifest diabetes may start years before actual diagnosis, i.e. often early in childhood. So the next question is what causes stress in young children. Severe life events in the family will most probably inflict psychological stress even in very young children, despite the fact that they may not even understand what is going on. But more common sources of stress may include maternal insecure attachment patterns leading to maternal insensitivity, and parenting stress, which may lead to temporary maternal insensitivity and other forms of tension in the child-mother relationship. Next, I would like to describe some of the relevant sources of stress in young children, but first what is stress?

Psychological stress

The stress concept

During the first half of the 20th century Cannon discovered that pain, fear, and rage are different types of psychological stress that physiologically increase the activity in the sympathetic nervous system and the production of adrenaline in the cortex. Cannon argued that the aggressiveness and fear are reactions to external threat and danger and named this the *fight-flight* reaction (Cannon, 1915, 1939). Starting during the 1930's, Selye expanded on this stress theory and defined stress as the result of any demand upon the body, whether the effect was mental or somatic, where the resources to cope were insufficient. According to Selye, distress (i.e. grief, resignation, and despair) physiologically increases the production of cortisol and stimulates a *conservation-withdrawal* reaction (Selye, 1936, 1980). Cannon's fight-flight reaction is a state of sudden arousal whereas Selye's conservation-withdrawal reaction is a state of inactivity and reduced energy spending associated with helplessness and/or hopelessness. Selye's conservation-withdrawal reaction has been proposed to nonspecifically facilitate disease development.

In 1984, Lazarus and Folkman defined psychological stress as *"a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being"* (p.19). Concerning very young children a stable temperament, secure attachment, and access to the "secure base" may constitute the available resources.

Stress in young children

Besides stressors such as biological needs (e.g. hunger and pain) and frustration over physical limitations, parental moods and caregiver insensitivity might be two of the most important stressors early in childhood. There is, of course, different reasons why a mother may fail to be sensitive to her infant and also different degrees of insensitivity. Far-reaching insensitivity, stemming from in-secure maternal attachment patterns, could probably be equaled to chronic stress in the infant. Whereas insensitivity for instance caused by parenting stress or serious life events may be more temporary. It has, for instance, been shown that maternal stress during infancy may sensitise children to later stress exposure (Essex et al., 2002).

Attachment

According to Bowlby's attachment theory, infants must maintain physical proximity to their caregivers for physical survival. In order to do so they need to be very sensitive to parental moods, signals and behaviors (Bowlby, 1958) and this is a biologically based adaptation to the range of likely caregiving environments (Main, 1990). The fact that this sensitivity is biologically based does not imply that it is always for the children's own good. Infants are biologically programmed to form attachment relationships even with parents who are dysfunctional (e.g. insecure) or abusive, at the cost of emotional stress and psychological vulnerability.

Maternal (caregiver) sensitivity (within the ABIS-project the mothers completed the questionnaires in most cases, therefore will "maternal/mother" mainly be used throughout the text) means that the mother will notice and attend to the child's signals in a non-distorted, empathic, and prompt way. This is the basis for the development of a secure infant-mother attachment relationship. The infant's emotional regulation is external at birth, i.e. comfort and satisfaction are received in the caregiving situation. During the second half of the first year the infant starts to internalize the predictability of the mother's behavior, and by the age of 1 year the child has developed a stable *internal working model* (IWM). This IWM includes a model of the experienced interaction, the availability and response of the attachment figure, and thereby also how worthy of attention and care (accepted/non-accepted) the child is. The IWM is relatively stable throughout life and will regulate, interpret, and predict the child's as well as other's (the attachment figure's) attachment-related behaviors, thoughts, and emotions.

The child of a sensitive caregiver, who notices the child's signals directly and attends to them in an empathic way, will develop a secure IWM. Such a child will also be able to use the caregiver, usually the mother, as a "haven of safety" or a "secure base" to return to in case of threat or danger, while exploring the environment (Ainsworth, 1967; Ainsworth et al., 1978). The knowledge of being worthy of care and love, and being cared for is the essence of attachment security, and since the IWM is relatively stable such a child will most likely grow up to become a sensitive parent. Attachment security in adults, characterized by a valuing of attachment relations, is an important foundation for one's personal resources.

On the other hand, children of mothers who are less sensitive in interactions, more interfering with the child's behavior, and less accessible to the children's bids are likely to develop insecure IWM:s. Mothers of *avoidant* infants are particularly striking in that they express an aversion to physical contact sought by

their infants, and express little emotion during interaction. Whereas mothers of *resistant* infants are especially oriented to fear in their infants, but interfere with the child's exploration and autonomy, and thereby promote dependency (Belsky, 1999). These infants are likely to develop *dismissive* and *preoccupied* IMW:s, respectively, as they grow up. They are also likely to experience repeated psychological stress during early childhood.

In adults, attachment insecurity can be of two different kinds either *dismissive*, characterized by a dismissive, derogating or detached attitude towards early attachment relations or *preoccupied*, characterized by an entangled, biased or mentally enmeshed attitude towards early attachment relations. Insecure maternal attachment patterns lead to maternal insensitivity (e.g. the mother being either dismissing of child attachment signals or self-preoccupied), which in turn causes psychological stress and insecure IWM:s in the infants, and this is how insecure IWM:s are transferred to the next generation.

The adult attachment interview (AAI) (Main, Kaplan, & Cassidy, 1985; Main, Goldwyn, & Hesse, 2001) is considered the best way to measure maternal attachment security predicting child outcomes. Insecure maternal attachment patterns measured with the AAI during pregnancy predicted insecure infant attachment classification at 1 year of age in 80% of the cases in a normal sample (Benoit & Parker, 1994). Significant secure-insecure differences in cortisol concentrations have also been found in 1-year olds after short child-mother separations (maximum 2x3 minutes), probably due to the fact that secure children are better able to cope with short separations than insecure children (Spangler & Grossmann, 1993). The implication of that study is that infants of insecure mothers are at risk of frequently repeated exposure to emotional stress and increased cortisol concentrations early in life.

It has been shown that insecure individuals are more prone to a wide range of negative outcomes ranging from less sensitivity in the interaction with their children to criminal behavior and psychopathology (e.g. Fonagy et al., 1997). Furthermore, insecure attachment patterns have been linked to a number of negative effects on cardiovascular, endocrine, and immune systems in adults and children (Maunder & Hunter, 2001). Finally, insecure individuals are more prone to stress (Nachmias et al., 1996; Luecken, 1998) and less likely to seek and benefit from social support compared to secure individuals (Florian, Mikulincer, & Bucholtz, 1995; Ognibene & Collins, 1998).

Parenting stress

Parenting stress has been conceptualized as a condition where the different aspects of parenthood result in a perceived discrepancy between situational demands and personal resources (Östberg, 1999). The parenting stress instrument used in the current study, the *Swedish Parenthood Stress Questionnaire* (SPSQ), based on the American self-report inventory *The Parenting Stress Index* (Abidin, 1990), concentrates on the parents' experienced stress in relation to their own parenting situation.

Parenting stress has been associated with a number of negative consequences, both for the children and for the parents. High levels of parenting stress have been linked to suboptimal maternal attitudes and mother-infant interactions, insensitivity to infant cues, and insecure infant-mother attachment relationships (Crnic, Greenberg, & Slough, 1986). Furthermore it has been suggested as an antecedent of child abuse (Rodrigues & Green, 1997). Concerning child behavior, parenting stress has been associated with more child externalizing (acting out) behaviors (Creasey & Jarvis, 1994) and more caretaking hassles, including poor infant sleep, feeding difficulties, excessive crying, cholic, and more childhood infections (Östberg, 1998). Parents' psychological well-being and the marital quality were both negatively affected by parenting stress (Lavee, Sharlin, & Katz, 1996).

There seems to be evidence enough to infer that parental stress affects and induces stress in the child, severely enough to affect the child's well-being negatively.

Serious life events

Holmes and Rahe (1967) were the first to offer a rating scale (The Social Readjustment Rating Scale, SRRS) tapping the magnitude of different life events, and not only the number and types of events. They argued that the greater the magnitude of a life change, the greater the probability that the life change would be associated with disease onset. Nevertheless their list of 43 weighted life events was criticized both on methodological grounds and concerning the usefulness of generalized magnitude ratings of life events. However, according to Dohrenwend and her associates, "the balance of evidence suggests that useful information is gained when life events are assigned different weights" (p.206) and that generalized (rather than individual post-hoc) ratings are needed in order to answer etiological questions (Dohrenwend et al., 1978). They suggested a method for scaling life events (handling the methodological problems in the SRRS) and created a new rating scale for life events – The PERI Life Event

Scale. This rating scale included 102 items and was based on sound methodological grounds.

The Swedish studies that have addressed life events in association with the development of type 1 diabetes (Thernlund et al., 1995; Hägglöf et al., 1994; Hägglöf et al., 1991) have all used modifications of Coddington's scale, which was designed to assess serious life events thought to be significant as etiological factors in childhood diseases (Coddington, 1972). Different methods were used, in the three Swedish studies, concerning the decision of negativity/severity/stressfulness of the life events. For example, 27 of the 40 items were decided by the authors to constitute negative events, based on earlier research (Thernlund et al., 1995).

Due to the already extensive questionnaires in the ABIS-study, the following seven serious life events were assessed: divorce, mother subjected/exposed to violence, serious disease in the family, serious accident in the family, loss of relative, becoming unemployed, and spouse/common-law spouse becoming unemployed. According to attachment theory, divorce and violence directed against the mother could be regarded as a blow to the family triad, including a threat concerning the child's access to his or her secure base (usually the mother), and a potential loss of or separation from one of the caregivers (usually the father). Circumstances like these are indeed very stressful for small children. Serious diseases or accidents within the family, and loss of relatives are other negative life events, which probably affect maternal sensitivity temporarily and could therefore constitute a negative life event for the child, even if they do not represent a threat to the child's safe haven. Table 2 shows the weights assigned to the serious life events measured in the ABIS-study (or their equivalent) according to four other studies.

Table 2: *Magnitude/weights of some life events according to different studies.*

Life event	The PERI Life Event Scale Dohrenwend et al. (1978) Scale: 1-100, where 1 is the most severe	The SRRS Holmes & Rahe (1967) Scale: 100-1, 100 is the most severe. (the rank)	Thernlund et al. (1995)	Hägglöf et al. (1991)
Loss of:				
Child	1 (negative)	-	-	-
Spouse	2 (negative)	-	-	-
Other family member	25 (negative)	-	-	-
Close family member	-	63 (5)	-	-
Mother	-	-	Negative	Loss/threatened loss in the family
Father	-	-	Negative	Loss/threatened loss in the family
Sibling	-	-	Negative	Loss/threatened loss in the family
Grandparent	-	-	Negative	Loss/threatened loss in the family
Divorce/Separation				
Divorce	5 (negative)	73 (2)	*Not* negative	Loss/threatened loss in the family
Separation of the parents	-	-	Negative	-
Illness/Injury				
Physical illness	3 (negative)	-	-	-
Injury (accident)	12.5 (negative)	-	-	-
Personal injury or illness	-	53 (6)	-	-
Serious illness or injury of the mother	-	-	Negative	Loss/threatened loss in the family
Serious illness or injury of the father	-	-	Negative	Loss/threatened loss in the family
Serious illness or injury of the child	-	-	Negative	-
Serious illness or injury of a sibling	-	-	-	Loss/threatened loss in the family
Unemployment	-	-	-	-
Laid off/Fired	50 (negative)	47 (8)	*Not* negative	*No* loss/ threatened loss in the family
Exposure to violence				
Assault	52 (negative)	-	-	-

Social support

Stress has been defined as the discrepancy between external stressors and personal resources and receiving adequate social support is one way to increase the personal resources, and thereby diminishing the gap to perceived demands (Crnic & Booth, 1991; Crnic & Greenberg, 1990).

Since numerous studies have provided evidence for beneficial effect of social support on well-being, Cohen and Ashby Willis investigated if support only had a buffering effect for individuals under stress of if it had a positive effect irregardless of stress (Cohen & Ashby Willis, 1985). Their review concluded that there is evidence for both models. They found evidence for a buffering model when the social support was assessed as the perceived availability of interpersonal resources to handle the needs elicited by stressful events. Evidence for a main effect model was found when support was assessed as a person's degree of integration in a large social network (Cohen & Ashby Willis, 1985).

Social support has for instance been shown to have a buffering effect on parenting stress, which was associated with lower role satisfaction and maternal self-esteem and with more extensive psychological and somatic symptomatology under low support conditions (Koeske & Koeske, 1990). Furthermore, social support was the only direct predictor of all assessed dimensions of parenting stress in Östberg's study (1999) and it had a reducing effect (Östberg & Hagekull, 2000).

Uchino, Cacioppo and Kiecolt-Glaser reviewed 81 studies in order to investigate the evidence for a relation between social support and health (1996). According to these authors both the quality and quantity of social relationships have been reliably related to morbidity and mortality, to the extent that lack of social relationships are comparable with standard risk factors including smoking, blood pressure, and physical activity. Social support was associated with beneficial effects on aspects of cardiovascular, endocrine, and immune systems.

Aims of the thesis

My **overall aim** has been to investigate whether psychological stress causes type 1 diabetes. However, it was not possible to investigate the association with manifest type 1 diabetes within the present thesis, due to the starting point of the ABIS-study (i.e. too few target-children so far). Thus, psychological stress was investigated in relation to diabetes-related autoimmunity.

Hence the **main aim** of the present thesis was to investigate whether psychological stress could be involved in the induction of diabetes-related auto-immunity. Using diabetes-related autoimmunity as the target variable was also advantageous since parental responses were not biased by awareness of their child's autoantibody status.

Research questions

In order to investigate this aim, a stress hypothesis was suggested (paper II) and some research questions were formulated.

1) What kind of stress should be studied in association with the induction of diabetes-related autoimmunity? (This issue has already been addressed in the Introduction)

2) Could psychological stress be involved in the induction of diabetes-related autoimmunity?
   a) Parenting stress? (paper IV)
   b) Attachment insecurity? (paper V)
   c) Serious life events? (paper VI)

3) Is medical screening, like the ABIS-study, in itself a cause for worry? (Paper I)

4) Does psychological stress induce beta-cell stress or an enhanced general immune response? (Study IV)

Some **methodological questions** have also arisen during the process:

5) What are the correlates of parenting stress? (Paper II and III)
   a) Could parenting stress be assessed with 6-point forced-choice Likert scales?

6) Are crude yes/no-measurements of social support and confidence/security useful? (Paper II, III, and IV)

7) Is a crude yes/no-measurement concerning experiences of unspecified serious life events useful? (Paper II, III, IV, and VI)

METHODS

All papers in the current thesis are based on data from the ABIS-project. ABIS stands for All Babies In Southeast Sweden and was initiated by Professor Johnny Ludvigsson at the Department of Molecular and Clinical Medicine, Division of Pediatrics, Faculty of Health Sciences, Linköping, Sweden.

Design

The ABIS-project

ABIS is a screening program for prediction of type 1 diabetes and other auto-immune diseases in the general child population. It was designed as a prospective cohort study aimed to follow all babies born in Southeast Sweden during Oct 1st 1997 and Oct 1st 1999 until school age.
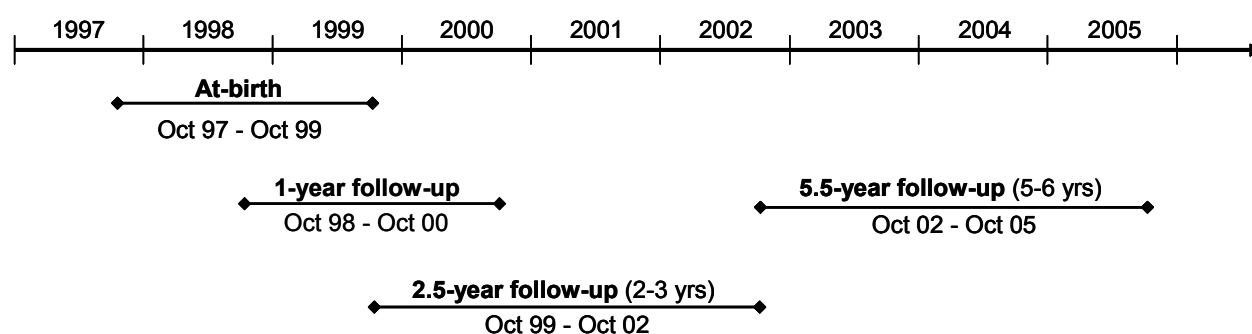
Figure 1: *The time table for the ABIS-project.*

Four time points for data collection were scheduled: at birth, 1 year, 2.5 year, and 5.5 year (Figure 1). A number of biological samples, mainly from the children, as well as questionnaire data were collected at each time point (Figure 2). The data collection was conducted by nurses at the 250 well child clinics in the region. The ABIS-data were collected in connection with the four compulsory childhood check-ups at the well child clinics, i.e. at 1 week, 1 year, 2-3 years, and 5-6 years of age.
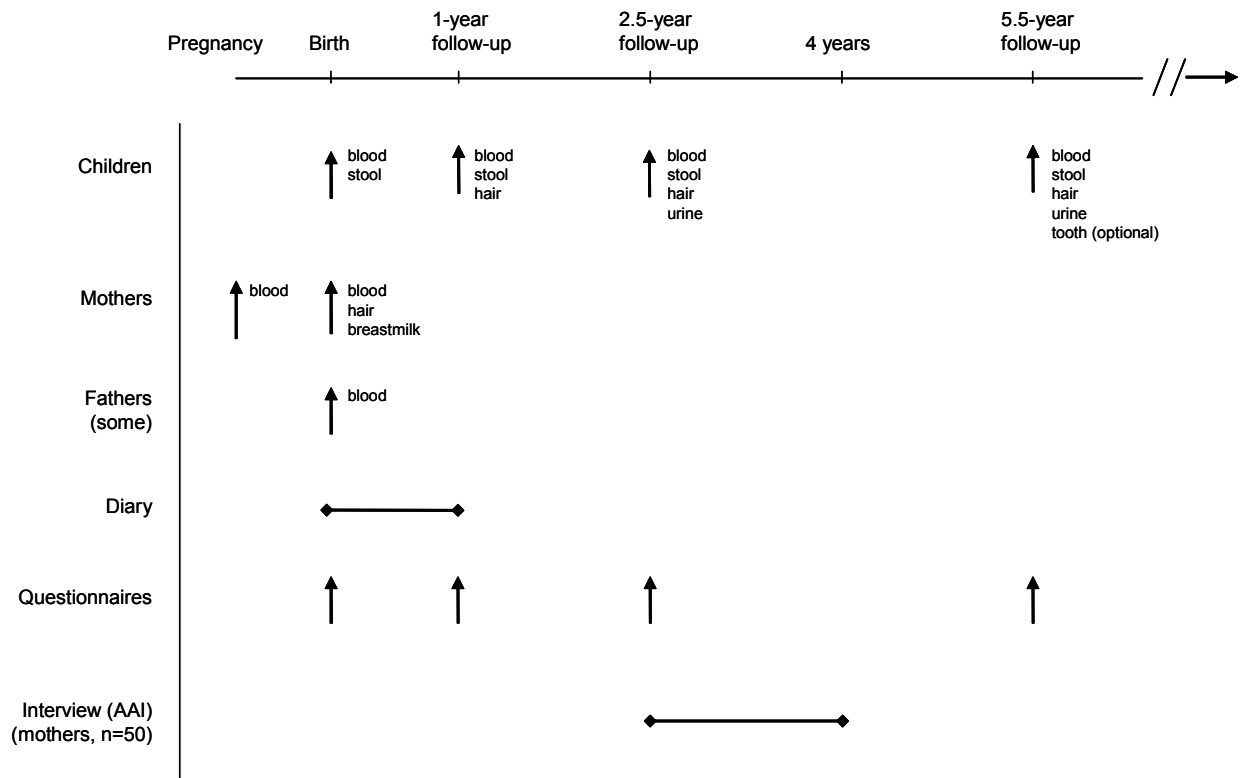
Figure 2: *The design table for the ABIS-project.*

The psychological part of the ABIS-project

I was engaged in this longitudinal project in June 1999 and at that time most of the at-birth questionnaires were already collected, the 1-year follow-up had been going on for half a year, and the 2.5-year follow-up was to start in half a year. Professor Ludvigsson had contacted Professor Ann Frodi in the spring of 1997 and invited her to be responsible for the psychological part of this large medical screening project. Below is a table of all psychological measurements included in the ABIS-questionnaires, some of the data were analyzed within the studies included in the present thesis (marked with an *), but most of the data from the 2.5-year follow-up are not yet analyzed and the 5.5-year follow-up is still in progress (Table 3). The wording and the original Swedish wording of some of these items can be found in Appendix 1.

Table 3: *Psychological measurements included in the different follow-up questionnaires of the ABIS-project.*

Measure-ments	At birth	1 year	2.5 years	5.5 years
Attitude towards partici-pation	1 item*; 5-point Likert Scale	1 item*; 6-point Likert Scale	1 item; 6-point Likert Scale	1 item; 6-point Likert Scale
Social support	1 item*: yes/no	-	1 item; 6-point Likert Scale	1 item; 6-point Likert Scale
				2x10 items, tapping quantity and quality of social network and emotional support. The instrument was constructed by the developmental psychology research group at the University of Uppsala.
Confidence /security	1 item*: yes/no	-	1 item; 6-point Likert Scale	1 item; 6-point Likert Scale
Serious life events	1 item* (mother): yes/no during pregnancy	1 item* (child during 1st year of life): yes/no and an open ended probe	1 item* (mother): yes/no, 7 fixed alternatives and an open ended probe	2 items (child & mother): yes/no, 7 fixed alternatives and an open ended probe
Parenting stress	-	SPSQ*: 34 items (Östberg, Hagekull, & Wettergren, 1997); 6-point Likert Scale	SPSQ: 34 items (Östberg et al., 1997); 6-point Likert Scale	SPSQ: 23 items (3 subscales: incompetence, role restriction, and spouse relationship problems) (Östberg et al., 1997); 6-point Likert Scale
Parental satisfaction	-	3 items* concerning different time points; 6-point Likert Scale	3 items concerning different time points; 6-point Likert Scale	-
Attachment security	-	-	AAI* (George, Kaplan, & Main, 1985) with selected mothers (child age: 2-4 years)	Possibly AAI (George et al., 1985) with selected mothers
			RSQ, 18 items (Griffin & Bartholomew, 1994)	
Child temper-ament	-	-	TBQ; 7 items (Hagekull, Lindhagen, & Bohlin, 1980)	-

* Data analyzed within the studies included in the current thesis.

Participants

Participants in the ABIS-project

All 21 700 parents-to-be in Southeast Sweden during Oct 1st 1997 and Oct 1st 1999 were invited to participate in the ABIS-project. 78.6% agreed to participate, yielding a sample of approximately 17 000 families at birth of which 16 058 completed the at-birth questionnaire. Approximately 13 700 families participated in the 1-year follow-up (10 836 completed the 1-year questionnaire), and approximately 10 700 participated in the 2.5-year follow-up (8 737 completed the 2.5-year questionnaire). Return of biological samples (in addition to cord blood) and/or a completed at-birth questionnaire was considered as participation.

The proportion of parents born abroad in the study cohort was similar to the proportion of individuals born abroad in the province of Östergötland, and only slightly smaller than the proportion in Sweden as a total (Table 4). Moreover, the sample was representative of Sweden concerning the parents' education.

Table 4: *Representativity of the ABIS-sample compared to Östergötland and Sweden, as well as follow-up rates within the ABIS-sample for some important variables.*

	SCB (Statistics Sweden, 1999)	ABIS: At birth (*n*=16,058; 100%)	ABIS: 1 year (*n*=10,836; 67%)	ABIS: 2.5 years (*n*=8,737; 54%)
Born abroad	Östergötland: 9% Sweden: 11%	F: 7% M: 7%	F: 6% M: 6%	F: 5% M: 6%
Education				
9-year compulsory school	F (25-34yrs): 12% M (25-34yrs): 14%	F: 9% M: 14%	F: 7% M: 14%	F: 7% M: 13%
Upper secondary education (≤3yr)	F (25-34yrs): 57% M (25-34yrs): 57%	F: 60% M: 62%	F: 60% M: 62%	F: 59% M: 62%
Third level education (SCB: <3yrs; ABIS: 1-3yrs)	F (25-34yrs): 20% M (25-34yrs): 20%	F: 19% M: 12%	F: 20% M: 12%	F: 21% M: 13%
Third level education (SCB: ≥3yrs; ABIS: ≥3.5yrs)	F (25-34yrs): 11% M (25-34yrs): 9%	F: 12% M: 12%	F: 13% M: 12%	F: 13% M: 12%
Parental age at birth (mean ± SD)	-	F: 29.6 ± 4.6 M: 32.0 ± 5.5	F: 29.8 ± 4.5 M: 32.2 ± 5.4	F: 29.8 ± 4.5 M: 32.2 ± 5.3
Single motherhood at birth	-	*n*=340; 100%	*n*=166; 49%	*n*=115; 34%
Lack of social support at birth	-	*n*=122; 100%	*n*=61; 50%	*n*=47; 39%
Lack of confidence/security at birth	-	*n*=54; 100%	*n*=29; 54%	*n*=21; 39%
Parenting stress at 1 year (mean ± SD)	-	-	2.54 ± 0.59	2.53 ± 0.60
Serious life events at 1 year	-	-	*n*=433; 100%	*n*=296; 69%

F=Female, M=Male

There were no differences between the original ABIS-cohort and the follow-up samples concerning: foreign origin, education, and parental age (Table X). Furthermore, there were no difference concerning parenting stress between the 1-year and the 2.5-year follow-up, and 69% of the children that had experienced a serious life event during the 1st year of life still remained in the study at the 2.5-year follow-up. The follow-up rates concerning single mothers at birth, lack of support, lack of confidence/security were more problematic, ranging from 49-54% at 1 year and 34-39% at 2.5 years.

Subsamples used in the empirical studies

Due to the enormous process of collecting and scanning the questionnaires, and the time-consuming process of analyzing blood samples, the number of cases available for statistical analyses steadily increased during the process of writing the articles included in this thesis. Data from the 2.5-year follow-up have also become available during this process (summer 2003).

The questionnaires were scanned and the blood samples were analyzed in the order they arrived from the well child clinics, i.e. no specific selection was made.

Paper I was based on the first 10 868 at-birth questionnaires available for statistical analyses.

Paper II included the first 4337 children from whom at-birth and 1-year follow-up questionnaires were available to statistical analyses.

Paper III, analyses of social support and confidence/security were based on all 16 058 at-birth questionnaires, and analyses of parenting stress were based on all at-birth and 1-year follow-up questionnaires, where more than 29 of the 34 parenting stress questions had been answered (*n*=10 304).

Paper IV was based on the first 4400 children from whom questionnaires and blood samples from the 1-year follow-up were available for statistical analyses.

Paper V was based on a small group of children consisting of approximately two autoantibody negative controls selected for each autoantibody positive child. 53 mothers of GADA-positive and 95 mothers of GADA-negative infants were randomly selected from the sample of 4400 families used in paper IV. They were invited to participate in the Adult Attachment Interview (AAI), and 18 mothers of GADA-positive infants vs. 32 mothers of GADA-negative infants accepted and were interviewed.

Paper VI was based on the first 5986 children from whom questionnaires and blood samples from the 2.5-year follow-up were available for statistical analyses. Paper VI also included a longitudinal follow-up of children who neither had autoantibodies nor had experienced a divorce at 1 year of age. This follow-up sample was based on the first 1845 children from whom questionnaires and blood samples were available for statistical analyses from the 1-year and 2.5 year follow-ups.

Procedures

Questionnaires and blood samples

The at-birth questionnaire was given to the mothers when they left the hospital following delivery. The questionnaire was filled out and returned either immediately or to the well child clinic at the first check-up (at one week of age).

Capillary blood was drawn from the child and a questionnaire was given to the parents at the 1- and 2.5-year check-ups at the well child clinics. The questionnaires were returned immediately or mailed back when completed. Some well child clinics reminded parents (by phone) who had not returned the questionnaire, and some did not. All parents who returned an incomplete 1-year questionnaire were contacted and asked to complete the full questionnaire. This procedure was not used concerning the 2.5-year questionnaire. 92% of the 1-year and 96% of the 2.5-year questionnaires were completed by the mother.

Blood samples were sent to the Clinical Research Centre, Faculty of Health Sciences, Linköping, where laboratory analyses of autoantibodies towards Tyrosine phosphatase (IA-2) and Glutamic Acid Decarboxylase (GAD) were done. The analyses of antibodies towards Tetanus Toxoid used in paper IV were performed.

Adult Attachment Interviews

The 50 mothers, who accepted the invitation to participate in the AAI interview, were contacted and an appointment was made. The interviews were conducted either at the hospital in Linköping or in the home of the mother. Interviews were carried out when the child was between 2.5 and 4 years of age, yielding an interview collection period starting spring 2000 and ending fall 2003. All interviews except one were conducted by the author. This exception was made due to a personal acquaintance between me and the mother. Neither the mothers

nor the interviewer was aware of whether the children were autoantibody positive or negative.

No reminders were used concerning the interview request, due to the risk of irritating or tiring parents and thereby losing participants in the ABIS-project.

Measurements

The extensive ABIS-questionnaires (117-196 questions) cover a large number of different topics including medical issues, environmental factors, nutrition, demographics, psychosocial situation, and psychological variables. Relevant to the papers in the current thesis were measurements of: attitudes toward participation, parenting stress, attachment security, social support, confidence/ security, serious life events, and a number of potential confounding factors.

Parenting stress

The Swedish Parenthood Stress Questionnaire (Östberg et al., 1997) was used at 1-year to measure parenting stress. The SPSQ is a Swedish stress index based on the American self-report inventory PSI (The Parenting Stress Index), which is said to provide an estimate of the level of stress a parent is experiencing and helps to define the perceived sources of experienced stress (Abidin, 1990). Östberg et al. elaborated on this index, focusing on the parent domain, adding positively worded new items, and excluding non-salient items. SPSQ consists of 34 items, with 5-point Likert-type response scales in Östberg's version. The stress score is calculated as a mean value of all SPSQ-questions answered. The SPSQ has shown a good test-retest reliability (r=.89) over 30 days, with a non-significant correlation (r=-0.19) between the number of days between the first and the second response and the difference between the two SPSQ-scores (Östberg et al., 1997).

In the ABIS-questionnaire, the SPSQ was used with forced-choice 6-point Likert-scales, with values 1-6, giving a theoretical median of 3.5 and a maximum stress score of 6. The reason for using 6-point Liker-type scales was to force the responder to make a decision and thereby avoid casual marks in the middle. The stress index for each participant was calculated as a mean score for the items answered.

There have been some differences concerning the use of SPSQ-data in the different papers. In paper II the stress score was dichotomized into high stress (above the 90th percentile) and low stress (below the 10th percentile). In papers

III and IV on the other hand, the cut-off for high stress was sharpened to above the 95th percentile. The reason was a wish to capture a high stress group (i.e. a risk group) despite a tendency of floor effect using the 6-point scales. In paper III the high stress cut-off was used as the test value in one-way t-tests, and in paper IV the high stress group (above the 95th percentile) was compared to the rest of the sample (i.e. below the 95th percentile) in order to include as many participants as possible.

An inverted normal points transformation was made and used in order to handle the skewed SPSQ-distribution in the multiple regression analysis in paper III (for details see paper III).

Another difference concerning the use of SPSQ between papers II on the one hand and III and IV on the other, was the inclusion criteria. Having answered 32 of the 34 SPSQ-questions was required for inclusion in paper II, whereas having answered 29 of the 34 SPSQ-questions was sufficient for inclusion in papers III and IV. The reason for changing to 29/34 was to assure the inclusion of single mothers, since the SPSQ contains a subscale of 5 questions concerning spouse relationships. 10 391 (95.9%) passed the 32/34 criterion whereas 10 514 (97.0%) passed the 29/34 criterion in the complete 1-year sample (*n*=10 836). However, this modification neither changed the mean score, the 90th, nor the 95th percentile when the complete 1-year sample was considered.

Attachment security

Maternal attachment security was assessed using the Adult Attachment Interview (Main et al., 1985) in a small group of 50 mothers (child age: 2-4 years). The AAI is a semi-structured in-depth interview aimed to reflect an individual's internal working models concerning attachment relationships. The mother's own childhood is discussed and the interview is said to "surprise the unconscious" in so far that episodic details are requested for semantic descriptions of the childhood experiences.

The interviews were tape recorded and transcribed verbatim. The transcripts were made non-identifiable and coded in random order during a time period of 8 months (April to October 2003). The coding, made bottom-up along 17 scales and top-down, according to the extensive manual (Main et al., 2001), leads to a main classification of a *secure*, *insecure-dismissing*, or *insecure-preoccupied* stance (internal working model) regarding attachment relationships, i.e. a personality structure. All 50 interviews were primarily coded by me. Interviews difficult to classify (*n*=7) were also coded by Professor Ann Frodi and a

consensus decision regarding classification was obtained. Inter-coder reliability concerning main classification has not yet been determined.

Certification for coding reliability, achieved through participation in a two-week training institute and successful completion of the one and a half year of reliability training, is required to use this coding scheme. I received my certification as a reliable coder in November 2001.

Serious life events

Serious life events were measured at 1 year using the following yes/no question: "Has the child experienced a serious or dramatic life event (e.g. death, divorce, new caregiver, or something similar)?", followed by an open ended question concerning the type of event: "if yes, what/which?". In the longitudinal follow-up (in paper VI) of children who neither had autoantibodies nor had experienced a divorce at 1 year of age this open ended question was used to exclude the 47 children who had experienced a divorce at 1 year of age.

Maternal experiences of serious life events were measured in the 2.5-year follow-up using the following yes/no question: "Have you experienced anything that you consider a serious life event since your child was born?", followed by seven structured alternatives to determine specific types of serious life events. The seven alternatives were divorce, subjected/exposed to violence, serious disease in the family, serious accident in the family, loss of relative, becoming unemployed, and spouse/common-law spouse becoming unemployed.

Attitude toward participation

Attitude to participation was assessed at birth and at 1-year. Mothers answered a 5-point Likert scale at birth and a 6-point Likert scale at 1-year, ranging from "very much calmer" to much more anxious", regarding how they felt concerning the fact that their child was part of a large medical screening project.

Social support and Confidence/security

Social support as well as (attachment) security are two very broad and well studied concepts, and there exist a number of different instruments to assess them (Crnic et al., 1983; Main et al., 1985; Griffin & Bartholomew, 1994). But what is the essence of support and security? The interpretation of these concepts is probably unique to each responder. For instance, some studies of perceived child temperament have shown that it is the mother's *perception* that determines behavioral outcome (Frodi, Bridges, & Shonk, 1989). So in order to minimize

steering the participants in any direction we decided to use the following two broad questions to assess social support and confidence/security: "Do you experience enough support from your social environment for yourself and your newborn baby?" Confidence/security was also assessed at birth with a similar question: "Do you feel that you have enough confidence/security so that you can give yourself and your newborn child a good start?" Both questions were asked in the at-birth questionnaire and answered with yes or no.

It is important to note that the connotation of the Swedish word for "confidence/security" implies psychological health and even the flavor of attachment security. These rather crude measures were also used to assure capturing the individuals who truly lacked social support or confidence/security.

In paper II these two questions were collapsed into one variable called "support/confidence" assessing lack of support, lack of confidence, or lack of both.

Diabetes-related autoantibodies

Autoantibodies against Tyrosine phosphatase (IA-2A) and Glutamic Acid Decarboxylase (GADA) were assessed, in the blood samples drawn from the child at 1 and 2.5 year of age. The blood samples were analysed by a laboratory method as described by Savola et al (Savola et al., 2001). Positivity for IA-2A and GADA was determined as antibody concentrations above the 95th percentile for one-year and 2.5-year old healthy children, respectively. IA-2A and GADA have also been used as continuous variables to compare median values in different sub-groups.

In the 2nd international work-shop (Diabetes Autoantibody Standardisation Program, 2002) the specificity was 100% for IA-2A and 96 % for GADA assays and the sensitivity was 54% and 81%, respectively.

Non-diabetes related antibodies

Antibodies against Tetanus Toxoid (TT) were used as a non-diabetes-related control antigen to investigate a general immune response. TT-antibodies were analysed in the blood sample drawn form the child at 1 year of age, in a sub-group (*n*=721) of the study population. The selection criteria were 1) lack of support, lack of confidence, and/or high parenting stress (i.e. above the 95th percentile on SPSQ; *n*=404), and 2) social support, confidence/security, and low parenting stress (i.e. below the 5th percentile on SPSQ; *n*=317). For details concerning the laboratory method for detection of TT-antibodies, see paper IV.

The concentrations of TT-antibodies were dichotomized at different cut-offs (75th, 90th, and 95th percentile) regarding positivity, and also used as a continuous variable in the statistical analyses in paper IV.

Potential confounding factors concerning autoimmunity

The results in paper IV and VI concerning risk factors for autoimmunity the following potential confounding factors were controlled for: diabetes heredity, increased parental age, breastfeeding, child infections, caesarean section, and need for neonatal intensive care, since all of these are known risk factors for type-1 diabetes mellitus and might influence the presence of autoantibodies.

Diabetes heredity was assessed both in the 1-year and the 2.5-year questionnaires by asking who, if any, of the child's family members or grandparents had type 1 diabetes. This question was recoded into two variables (not overlapping), "Type 1 diabetes in the family" (yes/no) and "Type 1 diabetes in the extended family" (yes/no). Both of these variables were controlled for in papers IV and VI. However, only the 2.5 year assessment was used in paper VI.

The parents' age when the child was born was assessed in the at-birth questionnaire, by asking for the child's and the parents' personal identification numbers. These numbers were recoded into year of birth and the differences between year of birth for the child and the parents were calculated. In paper IV a cut-off at 30 years was used (i.e. above or below 30), whereas the mean values with standard deviations were used in paper VI.

The length, in months, of exclusive breastfeeding was assessed at 1 year. Exclusive breastfeeding meant that the child had only been fed breastmilk and water.

Three different measures of child infections were assessed at 1 year. The number of *gastro intestinal influenzas* and *infections treated with penicillin* during the first year of life was dichotomized into three or more compared to two or less. The number of *upper respiratory infections* was dichotomized into two or more compared to one or none.

Delivery mode was assessed at birth. Normal delivery vs. caesarean section was recoded into one variable, excluding all other types of problematic deliveries.

Need for neonatal intensive care (yes or no) was also assessed in the at-birth questionnaire.

Ethical considerations

Paper I clearly indicates that the vast majority of the mothers were not worried by participating in the ABIS-project.

The parents received oral and written information, and were offered to see a video film about the ABIS project before the child was born. Return of a completed at-birth questionnaire and/or biological samples (in addition to cord blood) was considered as an informed consent. Participation in the 1-year and 2.5-year follow-up was considered as an additional consent from the parents. Ethical questions have been studied in-depth in a certain sub-project within the ABIS project, by Dr. U. G. Stolt, in cooperation with Dr. P-E Liss, and Dr. T. Svensson from the Department of Health and Society at the University of Linköping (Stolt, 2003; Stolt et al., 2003).

The majority of the questionnaires have already been collected and the data are stored according to the law of personal registration, and permissions have been obtained. Data are stored without personal identification numbers, and analyses are made according to current rules and with due confidentiality.

The adult attachment interviews have been approved by the Research Ethics Committee in Linköping. Neither the mothers nor the interviewer were aware of group belonging. The subject of the interview was regarded as interesting by most of the participants. Mothers with a difficult history have been offered contact with a licensed family therapist with more than 15 year of experience, Professor Ann Frodi, who also had the chief responsibility for this part of the project.

The ABIS project and the studies presented in the present thesis were approved by the Research Ethics Committees of the Faculty of Health Science at the University of Linköping, Sweden, and the Medical Faculty at the University of Lund, Sweden.

Statistical issues and analyses

SPSS 10.0-11.5 was used.

Chi-2 analyses

Since the majority of the questions used yielded categorical (nominal) variables chi-2 (χ^2) analyses were used in all papers. Chi-2 analyses compare proportions by calculating the difference between observed and expected values in each cell of a cross-tabulation. The chi-2 test should not be used if the expected value is

less than five in more than 20% of the cells of the cross-table, or if the expected value is less than 1. Under these conditions Fisher's exact test be should used instead. Fisher's exact test demands a 4-cell table (i.e. that two dichotomous variables are cross-tabulated), but is not sensitive to small expected values.

Sample size, Type I vs. II errors, and significance levels

Statistical significance is affected by sample size and in large samples even very small differences become statistically significant (i.e. to much statistical power). Therefore, the statistical significances found were evaluated regarding their individual theoretical plausibility and concerning the uniformity of the patterns of results, since an expected pattern of results is more likely to indicate a true effect.

Mass significance is a phenomenon due to the fact that statistical analyses are based on probabilities. A common cut-off for significance is 5% ($p<.05$), which means that the probability of each difference found being due to chance is less than 5%. It also means that the risk of finding a false significant difference (a type I error) is 5%, theoretically implying that 1 of 20 analyses come out significant by chance. The risk of finding false significances is therefore increased with the number of statistical analyses made. One way to decrease the risk for false significances (type I errors) is to use $p=<.01$ or $p<.001$ instead, which decreases the risk to 1 of 100 and 1 of 1000, respectively. Unfortunately, such a change increases the risk for missing actual differences (type II error) in the data.

Papers II, IV, and VI were based on a larger number of chi-2 analyses. In paper II mass significance was avoided by only regarding $p<.01$ and $p<.001$ as statistically significant. In papers IV and VI the risk of missing actual differences was regarded as more problematic than the risk of finding false differences. Thus $p<.05$ was used, and differences found were investigated for whether they were theoretically expected and explainable.

Multiple linear regression analyses and factor analyses

The statistical analyses in paper III are the most complex in the present thesis. One objective of those analyses was to explore the multi-dimensional construct of parenting stress by identifying specific predictor variables. In order to do so multiple linear regression analysis was used. The stress index was tested for and transformed to normality, since it is a basic assumption of the multiple linear regression analysis. All background variables assumed to be relevant to parenting stress were combined in a factor analysis. There were three reasons for

this decision: 1) to group the variables that belonged together, 2) to assure scale level on the independent variables demanded by the multiple linear regression analysis, and 3) to solve the problem with intercorrelations among the independent variables before running the multiple linear regression. The factor loadings were saved and used in the multiple linear regression analysis of parenting stress, with the 'enter' procedure.

Logistic regression analyses

Logistic regression analyses have been used to explore the multi-dimensional constructs of social support and confidence/security in paper III and to differentiate among the risk factors for autoimmunity in paper IV.

A logistic regression analysis should be used to explore predictors and calculate Odds Ratios (OR) with 95% Confidence Intervals (CI), when the dependent variable is dichotomous (e.g. yes/no). OR:s were used as approximate measures of Relative Risk (RR), since the SPSS does not permit the calculation RR with CI. The difference between OR and RR is very small when the risks are small, and it is widely accepted to use OR to approximate RR under these conditions. An OR above 1.0 indicates increased risk, but the OR is not reliable if the CI includes 1.0 or if the CI is large. The Hosmer and Lemenshow test was used for model validation. If this χ^2-statistic is non-significant the regression model represents the data in a correct way (Neter et al., 1996).

In paper III logistic regression models were calculated to identify specific predictor variables of social support and confidence/security, and the OR:s were used as a measure of increased risk for *lack* of support and *lack* of confidence/ security.

Independent variables that intuitively belonged together were combined in scales. Factor analyses were not used in this case since too few of the variables in the at-birth questionnaire theoretically belonged together to benefit from such a procedure, and since the logistic regression procedure is sensitive neither to scale levels nor intercorrelations (Hair et al., 1998).

Two sets of logistic regressions per dependent variable were conducted based on different samples. The reason for this was to give the logistic regression procedure a realistic chance to predict *lack* of support and *lack* of confidence/ security despite expected extremely skewed distributions, and still use the complete 1-year sample. The first set was based on sub-samples including equally small groups of mothers with and without support as well as with and without confidence/security. The small groups of mothers *with* support and confi-

dence/security were randomly selected from a reference group including all mothers who perceived enough support, enough confidence/security, and who scored below the 5th percentile on the SPSQ. This set of models was expected to have good predictive ability, despite the risk of yielding interminable CI:s for the OR:s, due to the small sample and expected skewed distributions of some of the variables. The second set of models based on the complete 1-year sample was expected to have poor predictive ability for *lack* of support and *lack* of confidence/security, while the CI:s would be acceptable given the large *N*. The forward stepwise procedure was used to create the small models, and the predictor variables from these models were then run with the 'enter' procedure on the full sample.

In paper IV logistic regression models were calculated (forward stepwise) to differentiate among the risk factors for autoimmunity found in the bivariate analyses, by running them together in a multivariate model and approximating their relative risk.

Investigation of potential confounding factors

The results in paper IV and VI were controlled for diabetes heredity, increased parental age, breastfeeding, child infections, caesarean section, and need for neonatal intensive care, since all of these are known risk factors for type-1 diabetes mellitus and might influence the occurrence of autoantibodies.

A variable has to be significantly related *both* to the dependent ("disease") and the independent ("exposure") variable in order to constitute a potential confounding factor.

Different techniques were used. In paper IV potential confounding factors were analysed in relation to the dependent variable (autoimmunity). Potential confounding factors significantly associated with the dependent variable in bivariate analyses were included in the logistic regression model to differentiate their contribution. The sample was also stratified for diabetes-heredity and the bivariate relation between parenting stress and autoimmunity was examined in the absence of diabetes heredity. In paper VI, on the other hand, potential confounding factors were analysed for their association with the independent variable (life events). Potential confounding factors significantly associated with the independent variable were analysed concerning their association with the dependent variable (autoimmunity) to investigate whether they might constitute true confounding factors. If they were non-related to the independent or the dependent variable they could of course not explain the results.

Student's *t*-test and Mann-Whitney's *U*-test

Student's *t*-test (independent) is used to compare mean values between different groups and Student's *t*-test (one-way) is used to investigate whether the mean value of a specific group is statistically above a fixed test value decided by the researcher. Mann-Whitney's *U*-test is designed to compare median values between different groups.

In paper III Student's *t*-test (independent groups) was used to compare the groups of mothers experiencing support and lack of support, as well as confidence and lack of confidence, and Student's *t*-test (one-way) was used investigate whether mothers lacking support or confidence scored significantly above the high (risk) cut-off concerning parenting stress.

In paper IV Mann-Whitney's *U*-test were mainly used to compare general levels (median values) of autoantibodies between different psychosocial risk groups, and Spearman's rank correlation coefficients (ρ) were used to analyze inter-correlations between different dichotomised psychosocial factors.

In paper VI Student's *t*-tests were used in the analyses of potential confounding factors measured on scale level (parental age and length of breastfeeding).

RESULTS

Suggesting a stress hypothesis (Paper II)

In paper II, bivariate chi-2 analyses revealed that a number of earlier identified risk factors for type 1 diabetes were significantly associated with lack of support/confidence and/or with parenting stress (Table 5).

Table 5: *Correlations between various background variables on the one hand, and lack of social support/confidence and high parenting stress on the other.*

Background variables (measured at birth)	Lack of Support/-Confidence		High Parenting Stress	
	χ^2	*df*	χ^2	*df*
Experiences of serious life events	24.759[b]	1	*Ns*	
Increased age (mother, above 30yrs)	*Ns*		33.429[b]	1
Increased age (father, above 30yrs)	*Ns*		19.772[b]	1
Maternal infections during pregnancy	17.684[b]	1	21.480[b]	1
Caesarian section	10.639[a]	2	*Ns*	
Need for NICU	9.904[a]	1	*Ns*	
Born abroad (mother)	30.235[b]	1	*Ns*	
Born abroad (father)	47.480[b]	1	*Ns*	
Single parenthood at birth	51.587[b]	2	6.891[b]	1
Low education (mother)	16.525[a]	5	*Ns*	
Low education (father)	*Ns*		*Ns*	
Unemployed (mother)	37.416[b]	1	12.329[b]	1
Unemployed (father)	9.493[a]	1	*Ns*	
Smoking during pregnancy	30.946[b]	1	*Ns*	

[a] p<.01; [b] p<.001; *Ns* = Not significant

Testing the stress hypothesis

Could psychological stress be involved in the induction of diabetes-related autoimmunity? (Paper IV, V, and VI)

Parenting stress (paper IV)

High parenting stress was significantly associated with positivity of IA-2A at 1 year of age (Table 6). This association remained significant even in the sub-groups 1) without diabetes-related autoimmunity in the family (*n*=3915),

$\chi^2(1)=9.929$, $p<.01$, and 2) without type 1 diabetes in the family ($n=4291$), $\chi^2(1)=11.381$, $p<.001$.

Table 6: *Multivariate analysis of the factors significantly associated to IA-2A positivity in the bivariate analyses. (n=4400).*

Predictor variables	IA-2A positivity at 1 year			
			Logistic regression	
	χ^2	p	OR	CI
High parenting stress	11.133	<.001	1.8	1.2-2.9
Experiences of serious life events	5.988	<.05	2.3	1.2-4.0
Maternal unemployment during 1st year	4.743	<.05	-	-
Upper respiratory infection (≥2 times) in the child during 1st year of life	5.820	<.05	1.6	1.1-2.3

Concerning earlier identified risk factors for diabetes (of a psychosocial character) were experiences of serious life events during the child's 1st year of life and maternal unemployment (not maternity leave) during the child's first year of life significantly associated with positivity of IA-2A. Among the potential confounding factors investigated were upper respiratory infections (≥ 2 times) during the child's 1st year of life associated with positivity of IA-2A. A logistic regression model, based on theses four variables, included experiences of a serious life event, parenting stress, and upper respiratory infections as predictors (Table 6).

With regard to positivity of GADA, there were significant relations to foreign origin (i.e. not born in Sweden) of the mother and low maternal as well as parental education. However, the logistic regression model included only foreign origin of the mother and low education of the father as predictors (Table 7).

Table 7: *Multivariate analysis of the factors significantly associated to GADA positivity in the bivariate analyses. (n=4400).*

Predictor variables	GADA positivity at 1 year			
			Logistic regression	
	χ^2	p	OR	CI
Mother born abroad	9.616	<.01	2.1	1.3-3.3
Low maternal education	3.909	<.05	-	-
Low paternal education	7.323	<.01	1.6	1.2-2.3

Only upper respirator infection, of the confounding factors analysed, turned out to be associated with autoimmunity (IA-2A) at 1 year of age. However this potential confounding factor received the lowest odds ratio and was thereby the least important predictor of autoimmunity (IA-2A), in the multivariate regression model (Table 6).

Serious life events (paper VI)

In paper VI experiences of divorce were significantly associated with positivity of IA-2A at 2.5 years of age (*n*=5986). The association remained when investigated in a longitudinal follow-up sample of children who neither had experienced a divorce nor were autoantibody positive at 1 year of age (*n*=1845). In this subsample had 12.5% (*n*=5) of the children exposed to divorce (*n*=40) developed autoimmunity compared to 3.8% (*n*=68) of the non-exposed (*n*=1805), Fisher's exact test, *p*<.05. The odds ratio for autoimmunity after having experienced a divorce was 3.6 (CI: 1.4-9.6) in the longitudinal follow-up sample, and 1.8 (CI: 1.1-3.1) in the complete *n*=5986 sample.

Furthermore mothers' exposure to violence was significantly associated with positive concentrations of IA-2A and/or GADA (i.e. double/single positivity) in their children at 2.5 year of age, $\chi^2(1)=4.625$, *p*>.05. The odds ratio for induction of autoimmunity after mothers' exposure to violence was 2.9 (CI: 1.1-7.8) (paper VI).

An examination of the odds ratios for all different kinds of serious life events measured in paper VI shows a clear trend towards increased risk of IA-2A positivity and double/single positivity (i.e. IA-2A and/or GADA) at 2.5 years of age, even though most of these associations did not quite reach statistical significance.

None of the confounding factors analysed within the study turned out to be associated both with the serious life event and autoimmunity, indicating that they did not explain the results in paper VI.

Attachment insecurity (paper V)

A potential association between attachment insecurity and the development of autoimmunity was investigated in paper V.

Table 8. *Distribution of maternal attachment insecurity/security over positive vs. negative concentrations of autoantibodies against GADA and IA-2A in the child at 1 year of age.*

Maternal attachment classifi-cations	GADA			IA-2A		
	Positive n=18 (36%)	Negative n=32 (64%)	Fisher's exact test p	Positive n=9 (18%)	Negative n=40 (82%)	Fisher's exact test p
2-way			.309			.400
Insecure	6 (50%)	6 (50%)		3 (27%)	8 (73%)	
Secure	12 (32%)	26 (68%)		6 (16%)	32 (84%)	

The results showed that the proportion of children with insecure mothers was equal between the autoantibody positive and the negative group (Table 8). However, the proportion of children with secure mothers was smaller in the auto-antibody positive than in the autoantibody negative group. Generally higher concentrations (median values) of autoantibodies were also found in the group of children with insecure mothers. However, none of the associations presented in paper V reached statistical significance.

Is medical screening, like the ABIS-study, in it self a cause for worry? (Paper I)

A very small group of mothers became more or much more worried by partici-pating in a large medical screening project like the ABIS-study (Figure 3). It is noteworthy that when participants answered the same question but the response alternative was a 6-point forced-choice scale they chose to answer on the "calmer side" of the scale.

Attitudes towards participation (at birth)

Attitudes towards participation (at 1 year)
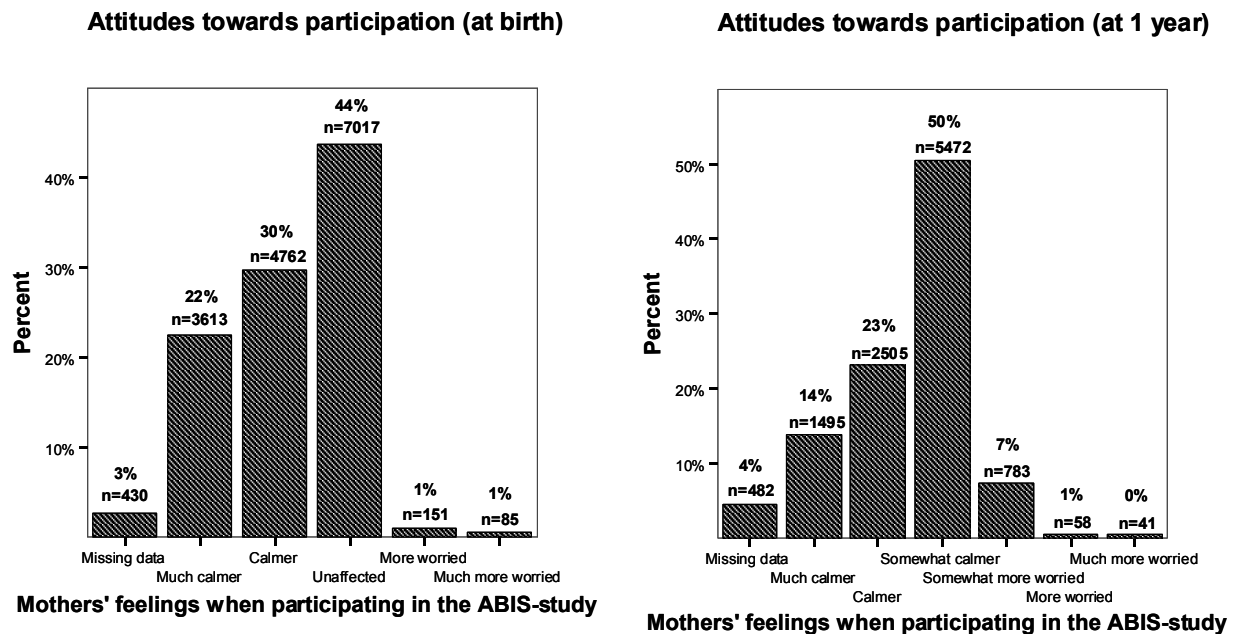
Figure 3: *Distribution of maternal answers concerning how they felt when their child participated in a large medical screening project. The results at 1 year have been recalculated based on the complete 1-year follow-up sample, compared to the results presented in paper I.*

Does psychological stress induce beta-cell stress or an enhanced general immune response? (Study IV)

In paper VI, concentrations of non-diabetes-related TT-antibodies were compared between psychologically defined high vs. low risk groups.

No significant associations were found between parenting stress, social support, and confidence/security on the on hand and enhanced immune responsiveness reflected as IgG-class antibodies towards Tetanus Toxoid on the other. Further more, neither IA-2A (ρ=.038, *ns*) nor GADA (ρ=.046, *ns*) correlated to TT-antibodies.

However, results from paper IV and VI (presented above) reveal a number of significant associations between different types of psychological stress and diabetes-related autoantibodies towards IA-2 and GAD.

Methodological questions

Correlates of parenting stress (Paper II and III)

Correlates to the construct of parenting stress were bivariatly analyzed in paper II and multivariatly analyzed in paper III.

Bivariate analyses in paper II showed that increased parental age, maternal infections during pregnancy, single motherhood at birth, and maternal un-employment during pregnancy were associated with high parenting stress (above the 90th percentile) (Table 5, above).

In paper III, fifty-two potential correlates from four different categories: socio-demographic variables, variables measuring maternal psychological issues, life style variables, and child characteristics, were included in the analyses, due to the multifaceted nature of family interactions. The 52 potential correlates were combined in factor analyses and the best solution contained 12 interpretable factors with a smallest eigenvalue of 1.24 and a cumulative explained variance of 43.2%. The final model included 4 factors concerning *child health* (factors 1, 3, 9, and 12); 2 factors concerning *child sleep patterns* (factor 4 and 11); 2 concerning *psychological variables* (factor 5 and 7); 1 regarding *consumption of sweets and junk food* (factor 6); and finally 2 factors regarding *demographics* (factor 2, 10, and 8) (Table III:2).

The factor loadings were saved and used in the multiple linear regression analysis (enter procedure) of parenting stress, revealing parental dissatisfaction and poor child sleeping patterns to predict parenting stress the best (Table 9). The correlation between parenting stress and 1) parental satisfaction (factor 5) was $r = -.399$, $p<.001$ and 2) child sleep quality (factor 4) was $r = .199$, $p=.001$.

Table 9: *Multiple linear regression analysis of Parenting Stress (SPSQ). n=3176.*

	Parenting stress (SPSQ)	
Predictors	Beta	*p*
Infectious diseases (Factor 1)	.033	.000
Age & no. of siblings (Factor 2)	.088	.024
Ear infections and Medication (Factor 3)	.053	.000
The Child's Sleep Quality (Factor 4)	.199	.000
Parental Satisfaction (Factor 5)	-.399	.000
Sweets and Junk food (Factor 6)	-.035	.017
Support & Confidence (Factor 7)	.048	.000
Educational level (Factor 8)	.010	.485
Allergic diseases (Factor 9)	.035	.017
Residence and Foreign origin (Factor 10)	-.010	.495
Bed & Waking-up times (Factor 11)	-.018	.218
Other diseases (Factor 12)	.033	.812
Maternal worries	.082	.000
R^2	.257	
Std. Error of the Estimate	.8288	
$F(13, 3162)$	84.277	
p	<.001	

Could parenting stress be assessed with 6-point forced-choice Likert scales?

Measuring parenting stress using the SPSQ with forced-choice 6-points Likert scales rendered a skewed distribution, with a tendency to floor-effect. The 95th percentile for the stress index in the complete 1-year sample (*n*=10 836) was 3.59, which is only slightly above the theoretical mean of 3.5, and the mean score in the complete 1-year sample was 2.59.

Are crude yes/no-measurements of social support and confidence/security useful? (Paper II, III, and IV)

Social support and confidence/security were measured with two crude yes/no-questions at birth in order to capture a true risk group, since it was assumed that it would take something extra ordinary to actually give a negative answer on a broad question like that.

Only 0.8% of the mothers (*n*=122) lacked social support and 0.4% (*n*=65) lacked confidence/security at the time of birth. Lack of support and lack of confidence/security were significantly related (Fisher's exact test p<.001).

Social support and confidence/security were combined into one variable in paper II and as can be seen in Table 5 (above) did the bivariate analyses reveal a number of significant associations namely: experiences of serious life events during pregnancy, maternal infections during pregnancy, delivery by caesarian section, need for neonatal intensive care, smoking habits during pregnancy and following demographic circumstances: born abroad, single parenthood, low maternal education, and unemployment.

In paper III multivariate logistic regression analyses were used to reveal predictors of social support as well as confidence/security. Concerning lack of social support was the following predictors found: lack of confidence/security (OR: 63.9; CI: 35.1-115.5), foreign origin (OR: 2.3; CI: 1.7-3.1), single motherhood (OR: 2.1; CI: 1.0-4.7), and maternal health problems (OR: 1.7; 1.4-2.1).

Concerning lack of confidence was the following predictors found: lack of support (OR: 81.5; CI: 44.5-149.0), serious life event (OR: 2.3; 1.2-4.4), maternal smoking during pregnancy (OR: 1.7; CI: 1.4-2.1), need for neonatal intensive care (OR: 1.6; CI: 1.2-2.2), maternal worries considering child health (OR: 1.4; CI: 1.2-1.7), and maternal health problems (OR: 1.4; CI: 1.0-1.9).

There were also significant relations between high (risk) parenting stress on the on hand and lack of support, $\chi^2(1)=58.973$, p<.001, as well as lack of confidence/security, $\chi^2 (1)=77.584$, p<.001 on the other. Mothers lacking social support, $t(54)=-5.134$, $p<.001$, or confidence/security, $t(10168)=-7.062$, $p<.001$, scored significantly higher on the parenting stress index than mothers with support or confidence/security, respectively. However, lack of social support and lack of confidence/security were overshadowed by other variables as predictors of stress in the multivariate analyses.

Paper IV revealed that neither lack of social support (for IA-2A: $\chi^2(1)=2.371$, *ns*, and for GADA: $\chi^2(1)=1.172$, *ns*) nor lack of confidence/security (for IA-2A: $\chi^2(1)= .422$, *ns*, and for GADA: $\chi^2(1)=2.151$, *ns*) was associated with induction of autoimmunity in the child at 1 year of age.

Is a crude yes/no-measurement of experiences of unspecified serious life events useful? (Paper II, III, IV, and VI)

Experiences of unspecified serious life events were measured with a yes/no-question at birth, in the 1-, and 2.5-year follow-up questionnaires. The question is now whether this type of crude measurement could be useful.

Having experienced unspecified but serious life events during pregnancy was associated with lack of support and/or confidence, $\chi^2(1)=24.759$, $p<.001$, in paper II and was revealed as a quite strong predictor (OR: 2.3; CI: 1.2-4.4) in the multivariate analyses of lack of confidence in paper III.

In paper IV experiences of unspecified but serious life events in the family during the child's 1st year of life increased the risk of diabetes-related autoimmunity in the child (OR: 2.3; OR: 1.3-4.0).

Only a slight non-significant tendency of increased risk (ORs: 1.1) of diabetes-related autoimmunity in the child at 2.5 years of age due to maternal experience of unspecified life events, were found in paper VI.

DISCUSSION

The discussion will start with a brief summary of the main findings and conclusions concerning the aims outlined in the introduction, followed by a discussion of theses issues. Thereafter, I would like to point out some limitations of the present research and make some suggestions for the future. Finally, I would like to highlight the strengths and uniqueness of the present research and propose some general conclusions.

Aims and main findings

The main aim of the present thesis was to investigate whether psychological stress could be involved in the induction of diabetes-related autoimmunity, as a step on the way in investigating whether psychological stress can cause Type 1 diabetes.

A stress hypothesis was suggested and some initial support was found. The main findings concerning the research questions formulated were:

What kind of stress should be studied in association with the induction of diabetes-related autoimmunity?

- According to the literature review in the introduction especially the role of caregiver insensitivity should be investigated concerning the induction of autoimmunity, since it is a prominent stressor in infancy. There are different reasons for caregiving insensitivity, including the parent's attachment insecurity, parenting stress, and experiences of serious life events.

Could psychological stress be involved in the induction of diabetes-related autoimmunity?

- Parenting stress was associated with the induction of diabetes-related autoimmunity at 1 year of age, even in children without diabetes heredity, and the result was not explained by any of the potential confounding factors.

- Attachment insecurity was not associated with the induction of diabetes-related autoimmunity at 1 year of age. However, this lack of association was probably due to methodological constraints of study V.

- Serious life events like divorce and maternal exposure to violence were associated with the induction of diabetes-related autoimmunity at 2.5 years of age. The risk of developing diabetes after parental divorce or mothers' exposure to violence was about threefold. None of the results were explained by any of the potential confounding factors.

Is medical screening, like the ABIS-study, in itself a cause for worry?

- The vast majority of the parents were calmed or unaffected concerning the participation in the ABIS-project, suggesting that large-scale screening-project in the general population can be performed without causing increased anxiety.

Does psychological stress induce beta-cell stress or an enhanced general immune response?

- A number of associations between psychological stress and diabetes-related autoantibodies were found, but none with antibodies towards Tetanus Toxoid. These results suggest that beta-cell stress, perhaps due to insulin resistance rather than an enhanced general immune response, seems to be the more promising link.

Main findings concerning the **methodological questions** posed in the introduction:

What are the correlates of parenting stress?

- Parental dissatisfaction and poor child sleeping patterns were the strongest predictors of parenting stress, and daily hassles like poor quality child sleep appeared to be more important than child health problems, which were assessed in detail.

Could parenting stress be assessed with 6-point forced-choice Likert scales?

- Assessing parenting stress, using the SPSQ with 6-point forced-choice Likert scales, rendered a negatively skewed distribution, suggesting that SPSQ should be used with 5-point non-forced-choice Likert scales as it was designed to, despite the risk of casual marks in the middle.

Are crude yes/no-measurements of social support and confidence/security useful?

- The results indicated that broad yes/no-questions regarding perceived support and perceived confidence/security do capture a small but true risk group. However lack of social support and confidence/security assessed with this type of measurement was not associated with diabetes-related autoimmunity in the child and they were overshadowed by other factors in the multivariate analyses.

Is a crude yes/no-measurement concerning experiences of unspecified serious life events useful?

- Experiences of unspecified, but serious, life events assessed with a crude yes/no-question appear to be associated with child outcomes such as diabetes-related autoimmunity during infancy. However no association with diabetes-related autoimmunity in the child using this type of measurement was found at 2.5 years of age.

Conclusions regarding aims and research questions

- Psychological stress is suggested to be associated with the development of diabetes-related autoimmunity, insofar that psychological stress may induce insulin resistance leading to beta-cell stress, which further could trigger an autoimmune reaction towards beta-cells in genetically pre-disposed children.

- A number of disparate risk factors for type 1 diabetes were associated with common psychological mechanisms, indicating that the suggested stress hypothesis needed further investigation.

- Psychological stress, *whether measured as parenting stress or exposure to negative life events*, seems to be involved in the onset of diabetes-related autoimmunity in young children. These findings strengthen the proposed stress hypothesis.

- Somewhat surprisingly, the associations between psychological stress and diabetes-related autoimmunity were not explained by any of the seven potential confounding factors analyzed, suggesting that psychological stress is an independent risk factor for diabetes-related autoimmunity.

- Whether psychological stress, measured as maternal attachment insecurity, is involved in the onset of diabetes related autoimmunity warrants further investigation.

- Psychological stress specifically affected diabetes-related beta-cell autoimmunity (i.e. beta-cell stress) rather than caused a general effect on the immune system, a finding that also supports the stress hypothesis.

- Psychological stress could be a risk factor for the development of type 1 diabetes, since it appears to be one trigger mechanism behind diabetes-related autoimmunity early in life.

- Large-scale screening studies for the prediction of diabetes in the general child population can be performed without causing increased anxiety.

- Although parenting stress has a complex origin certain risk factors are more crucial than others, such as dissatisfactions with the parenting role and child sleep problems. In fact, daily hassles like poor quality child sleep appear to be more important than even child health problems.

The stress hypothesis

The stress hypothesis as part of the beta-cell stress hypothesis, proposed by my supervisor Professor Johnny Ludvigsson (personal communication) during the planning of my dissertation studies, suggests that psychological stress via cortisol and insulin resistance may induce beta-cell stress that in genetically predisposed individual could trigger an autoimmune beta-cell destruction leading to Type 1 diabetes.

As a first step to investigate this hypothesis, common psychological mechanism were investigated concerning their association with certain environmental factors earlier linked to the development of type 1 diabetes in children. Some initial support for the stress hypothesis was found, given that following previously identified risk factors of type 1 diabetes: experiences of serious life events, increased parental age, maternal infections during pregnancy, delivery mode, foreign origin, single parenthood, low parental education, unemployment, and smoking habits during pregnancy, were associated with psychological mechanisms. Most of these factors, especially in combination, are well known to induce stress, so their associations with lack of social support and/or confidence/security at birth and high parenting stress at 1 year were not surprising.

These earlier identified risk factors for T1D are rather disparate from a biological point of view, and they might of course contribute to diabetes in various ways. However in paper II, psychological stress was suggested as a *common explanation* for these disparate risk factors (Figure 4). Maybe the use of the word "mediate" in the original proposition of the stress hypothesis in paper II was unfortunate, since the thought was to find a *common explanation*, rather than investigating interaction effects of psychological stress on these earlier identified risk factors.
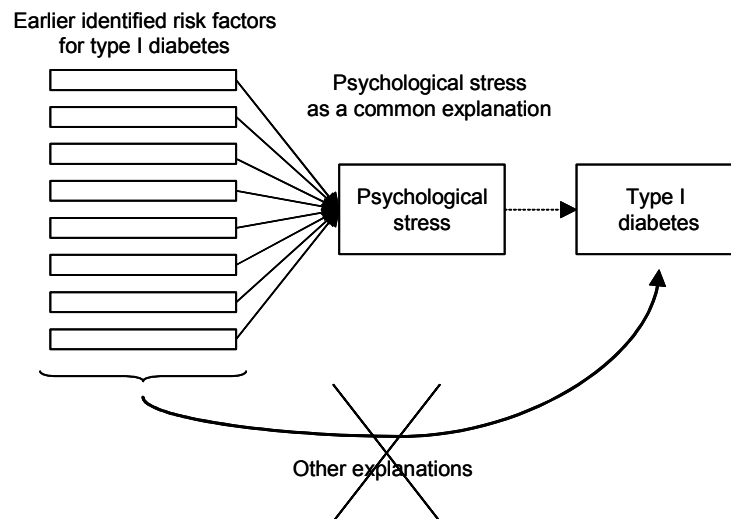
Figure 4. *Illustration of the thought of psychological stress as a common explanation for earlier identified, but disparate, risk factors for Type 1 diabetes as suggested in the stress hypothesis.*

Stress may induce
diabetes-related autoimmunity

In two of the empirical studies, based on prospective population-based samples, obvious psychological and psychosocial stress factors such as high parenting stress, foreign origin of the mother, low parental education, experiences of unspecified but serious life events, maternal employment, divorce, and mothers' exposure to violence were found to be associated with the induction of diabetes-related autoimmunity in young children.

High parenting stress, unspecified serious life events and psychosocial strain in the family, i.e. foreign origin, low education, and unemployment were associated with the induction of autoimmunity at 1 year of age. The association with high parenting stress remained even in the sub-samples without diabetes heredity. Experiences of parental divorce as well as if the mother had been exposed to

violence were associated with autoimmunity at 2.5 years of age. The risk of developing diabetes-related autoimmunity after parental divorce was approximately threefold in a longitudinal follow-up sample of children that neither had autoimmunity nor had experienced a divorce at one year of age. Mothers' exposure to violence did also increase the risk of the child developing autoimmunity about threefold.

These findings support and strengthen the stress hypothesis and are in line with earlier studies of stress as an etiological factor of Type 1 diabetes (Thernlund et al., 1995; Hägglöf et al., 1991; Vialettes et al., 1989). Furthermore, the findings suggest that psychological stress may be involved even in the *onset* of diabetes-related autoimmunity as proposed by Thernlund and her colleagues (1995), and not only precipitate manifest T1D as earlier shown (Hägglöf et al., 1991; Vialettes et al., 1989).

Moreover, these findings support the view that psychological stress in families induces stress in the child (e.g. Essex et al., 2002; Belsky, Lerner, & Spanier, 1984), which was not especially surprising considering the severity of the psychological circumstances. High parenting stress generates a risk of temporary maternal insensitivity and non-access to the secure base for the child. In addition to these threats, experience of divorce constitutes a blow to the family triad including loss of daily contact with one of the attachment figures. Furthermore, divorce or parental separation has earlier been judged as a very severe life event (Thernlund et al., 1995; Hägglöf et al., 1991; Dohrenwend et al., 1978; Holmes & Rahe, 1967) and has also shown to have negative long-term effects on the children (Wallerstein, Lewis, & Blakelsee, 2000).

However it was surprising that the stress was transferred to such a degree, i.e. severely enough to induce diabetes-related autoimmunity in genetically predisposed children.

Somewhat surprisingly, none of the potential confounding factors analyzed explained the association between psychological stress and diabetes-related autoimmunity.

However, no association between attachment insecurity and diabetes-related autoimmunity was found, probably due to methodological constraints of study V. Theoretically, one could still expect an association between attachment insecurity and induction of diabetes-related autoimmunity, and this issue warrants further investigation.

The interpretation of autoimmunity

High concentrations of diabetes-related autoantibodies circulating in the blood precede manifest Type 1 diabetes and are used to identify subjects at risk of developing the disease. However, using these autoantibodies to predict manifest diabetes is not straight forward. First of all the concentrations of these autoantibodies fluctuate in early childhood in the normal population (Kimpimaki et al., 2002). Secondly, the existence of autoantibodies in the blood is only a reflection of on-going beta-cell destruction and not a direct sign. A couple of other problems yet to be resolved include: 1) what autoantibody combination is best to use; 2) what concentration should be looked at; 3) and at what age are high concentrations most predictive.

The majority of the children who were positive for one of the diabetes-related autoantibodies at 1 or 2.5 years of age will never develop Type 1 diabetes. Consequently, the results in the empirical studies of the present thesis imply that psychosocial stress was only associated with the induction of diabetes-related beta-cell autoimmunity.

However, beta-cell autoimmunity in itself indicates that something is wrong with the immune system, since it attacks and destroys own healthy tissues (autoimmunity). A slight reaction towards an external antigen, or even a body-own antigen, could be a natural reaction if tolerance is developed as a result. But if someone develops a larger quantity of antibodies toward body-own antigens than 95% of all healthy children, it is definitely an abnormal reaction and something is wrong with the immune system of that child.

Beta-cell stress is a possible pathway

The topic of immunology was not the focus of the studies in the present thesis. However, the question whether the association between psychological stress and autoimmunity is mediated via beta-cell stress or via an enhanced general immune response is important for the stress hypothesis. Therefore, this question was briefly addressed in a subsample in paper VI, where concentrations of non-diabetes-related antibodies towards Tetanus Toxoid were compared between a psychologically defined high vs. low risk group.

The investigation of the biological pathway was incomplete. Nevertheless it seems reasonable to conclude that beta-cell stress, rather than a generally enhanced immune response, appears to be the more promising link between psychological stress and induction of autoimmunity. However, this issue definitely

warrants further investigation. Furthermore, in order to verify the stress hypothesis it will be important to exclude other possible pathways.

Screening is not a cause for worry

Type 1 diabetes is a common and very serious disease among children and the incidence is increasing in Sweden (Swedish National Register of Childhood Diabetes) and in the rest of the western world (Green et al., 2001; EURODIAB ACE Study Group, 2000). In order to learn how to prevent Type 1 diabetes, studies of etiological factors in the general population must be conducted. It is important to gain knowledge about factors involved in the onset of the autoimmune process, factors contributing to continued beta-cell destruction as well as salutary factors, which might stop or even reverse the autoimmune beta-cell destruction leading to Type 1 diabetes. When a child is diagnosed with manifest Type 1 diabetes, it is too late to intervene, since almost all beta-cells are destroyed and insulin can no longer be produced in the body.

If we are ever to prevent Type 1 diabetes in more than the small minority of children identified by a first degree relative with Type 1 diabetes, screening for genetic risk and diabetes-related autoantibodies in the general child population is also necessary.

According to the British Diabetic Association screening to predict Type 1 diabetes using a combination of autoimmune markers is not useful in the general population (Avery et al., 1998), and hence should not be conducted. Furthermore, they recommended that "screening {of first-degree relatives} should only be undertaken after full counseling and follow-up should be offered" (text in brackets included by the author, p. 643, Avery et al., 1998). However, the results of study I clearly indicated that this type of prospective screening in the general child population can be conducted without causing increased worry among the absolute majority of the parents. Results from the ABIS-project have also revealed that, the parents even want to know if their children have an increased risk of developing Type 1 diabetes, despite the fact that there is no intervention available at the time being (Stolt, 2003). However, we should be aware that a small group of parents do get worried and will need extra information and support.

Hopefully, increased knowledge about the trigger mechanisms and their complex interplay, gained through prospective population-based studies, will help to find ways to prevent some cases of diabetes in the future or give possibilities to intervene in high risk groups. At the present time, there are no

methods for prevention or intervention that have proven useful. Each case of Type 1 diabetes that could be prevented is of course of great human significance, but it is also of economical importance to society.

Correlates of parenting stress

Correlates of the construct of parenting stress were analyzed bivariately as well as multivariately. The bivariate analyses showed increased parental age, maternal infections during pregnancy, single motherhood at birth, and maternal unemployment during pregnancy to be associated with high parenting stress. Furthermore significant relations between high parenting stress and lack of support as well as lack of confidence/security were also found. However, all these variables were overshadowed in the multivariate analyses of parenting stress.

Included in the multivariate analyses of parenting stress were a large number of potential correlates from four different categories, including sociodemographic variables, variables measuring maternal psychological issues, life style variables, and child characteristics. The issue of child health was, for example, covered by 13 items over four different categories. However, the issue of child health was not included among the predictors revealed by the multivariate analysis. Instead, parental dissatisfaction and poor child sleeping patterns were found to be the best predictors of parenting stress. However, the correlations were rather weak (parental satisfaction: $r = -.4$ and child sleep quality: $r = .2$), and the explained variance was around 25% indicating that the largest part of the construct of parenting stress remains to be explained.

Despite these limitations and the fact that parenting stress is a complex phenomenon certain risk factors could be emphasized. Not surprisingly, parental dissatisfaction and poor sleep were found to predict parenting stress. Crnic and his colleagues have emphasized the close link between dissatisfaction with the parenting role and parenting stress (Crnic & Booth, 1991; Crnic & Greenberg, 1990) and the close association with poor sleep has also been shown earlier (Thunström, 1999). Östberg and her associates have underlined the issue of caretaking hassles as a predictor of parenting stress (Östberg & Hagekull, 2000). However, the current results suggest a differentiation of the issue, in so far that daily hassles like sleep problems seem to be more important than child health problems. Based on these results we would like to highlight the importance of sleep and sleep problems and suggest that well child clinic staff routinely discuss various coping strategies with parents in order to reduce parenting stress.

Part of the association between parenting stress and lack of social support found, is probably explained by the fact that the Swedish Parenting Stress Questionnaire (SPSQ) contains a subscale of seven items concerning social support specifically.

Unfortunately, the direction of effects between parenting stress, dissatisfaction, and poor child sleep could not be disentangled given the nature of the design in the current study.

Limitations and methodological issues

Indirect measures of child stress

One possible limitation of the current research is the indirect measures of child stress used. If the aim is to investigate the role of stress in the development of disease, one could argue that the stress as well as the disease should be measured within the same individual. However, this was not possible within the current studies. Furthermore, Belsky (1984) among others, have argued that the *whole* family perspective must be included when investigating stress in young children, given that young children are so receptive of parental moods and parental sensitivity. The family perspective is probably more important the younger the child is, because of the external emotional regulation in infancy and the fact that this regulation gradually internalizes during childhood.

One could also argue that if the biological link of interest is cortisol leading to insulin resistance, the children's cortisol concentrations should have been measured. However the use of cortisol to reflect stress is not straightforward. One problem is that cortisol only gives a momentary picture of the stress level and it is normally used to compare concentrations before and after some stressful event, where every subject is its own control. Cortisol concentrations measured in round-the-clock urine samples could perhaps have been used to indicate somewhat more chronic stress, but it is not easy to obtain analyzable round-the-clock urine samples from small children using diapers.

Difficulties with and absence of measurements

Parenting stress was assessed using the SPSQ with forced-choice 6-points Likert scales to avoid casual marks in the middle, but this left us with a negatively skewed distribution. Fortunately, the problem could be solved by using an inverted normal point transformation of the mean values. Yet, I would like to

recommend using the SPSQ with 5-point Likert scales as it was designed to (Östberg et al., 1997), despite the risk of casual marks in the middle.

The results from papers II, III, and IV suggests that a simple yes/no-question concerning experiences of unspecified but **serious life events** can be useful in the prediction of child outcomes such as diabetes-related autoimmunity in infancy as well as psychological states such as lack of confidence/security. However, concerning prediction of diabetes-related autoimmunity at 2.5 year of age this type of measurement was not as useful.

Experiences of unspecified serious life events were measured with yes/no-questions at birth and at 1 year. In the 1-year questionnaire, this question was followed by an open-ended probe allowing the mother to specify the type of event. An advantage with this type of measurement is that it was up to the mothers to decide if they had experienced anything that they themselves considered to be a serious life event. However, the issue of subjectivity was more problematic at 1 year, since the mothers decided the significance of events in the child's life. For example, a divorce could be judged as good and unproblematic by the mother, despite the fact that research has found negative long-term effects on children due to parental divorce (Wallerstein et al., 2000). Therefore, it would have been even better additionally to include a number of specified serious life events as response alternatives, followed by an open-ended probe, especially in the 1-year questionnaire.

The results suggested that a simple broad yes/no-question regarding perceived **social support** as well as **confidence/security** asked in connection with the delivery was sufficient to capture a small but true risk group, in so far that all the variables associated with lack of support and/or confidence/security in paper II makes sense theoretically. The multivariate results from paper III further supports this conclusion, since rather severe circumstances predicted lack of support as well as lack of confidence/security. The significantly higher parenting stress scores found in mothers' lacking support or confidence/security is also in line with this conclusion.

Despite the evidence that our crude measure of support and confidences captured a small but true risk group no association with the induction of autoimmunity in the child at 1 year of age were found. Therefore the conclusion must be modified: this type of crude measure does not seem to be useful concerning prediction of autoimmunity in infants. It would have been better to use validated instruments or at least allowing responses on Likert-scales. Measurements of support and confidence/security could perhaps also have been included

in the 1-year follow-up. However, the fact that the SPSQ contains a subscale of seven items tapping social support compensates to a certain extent the fact that social support was not separately measured at 1 year.

Finally, the absence of measurements assessing **the child's attachment security** and **temperament** at 1 year is unfortunate. Maternal attachment security could also have been assessed at birth.

Potential confounding factors

Interesting associations between psychological stress and autoimmunity were found in two of the empirical studies, and somewhat unexpectedly did none of the potential confounding factors analyzed explain the results. The question now is whether all potential confounding factors have been satisfactorily investigated. In the results of study IV and VI were following potential confounding factors controlled for: diabetes heredity, child infections, dietary components, increased parental age, caesarean section, and need for neonatal intensive care, since all of these factors earlier have been identified as risk factors for the development of Type 1 diabetes.

It was important to control for **diabetes heredity** since there is a 90% risk of developing Type 1 diabetes if one of the parents has the disease, but this measure does not control for genetic risk since only 1 in 10 newly diseased actually has a first degree relative with T1D (Dahlquist & Mustonen, 2000). However, no one single gene or even combination of genetic factors can accurately identify individuals at risk for Type 1 diabetes (Avery et al., 1998). It also seems quite far-fetched to think that parents of children who carry risk genes would be more prone to parenting stress or divorce than other parents, especially if they are unaware of their children's genetic constitution.

Regarding **viral infections**, frequencies of different groups of child infections were controlled for, i.e. upper respiratory infections, infections treated with penicillin, and gastro-intestinal influenzas. These kinds of measurements not specifically control for entero-viral infections, since antibodies or specific viruses were not assessed. Still, the questions asked give a certain control for infections, which should be sufficient for the conclusions drawn.

Dietary components, especially gluten and cow's milk, were controlled for by asking for length of exclusive breast-feeding. In Sweden exclusive breast-feeding during the first four months is strongly recommended (by the well child clinics, used by the vast majority of all new mothers), and according to the recommendations, food containing gluten should not be introduced before 6

months of age. At around 6 months of age, exclusive breast-feeding is no longer enough and additional food must be given. However, formula based on cow's milk could be given earlier than 4 months of age if necessary, and especially this fact was controlled by asking about exclusive breast-feeding. Exclusive breast-feeding has also been shown to have a protective influence on the subsequent onset of type 1 diabetes in childhood (McKinney et al., 1999).

Increased parental age, **caesarean section**, and **need for neonatal intensive care** were controlled for, but it was unfortunately not possible to control for excessive compensatory weight gain after low birth weight, since that would have demanded continuous information rather than questionnaires filled out at-birth and at 1 year. However, McKinney and her colleagues found neither associations between the development of diabetes and high- or low-birth weight (McKinney et al., 1999) nor small-for-date (McKinney et al., 1999; McKinney et al., 1997).

Given current knowledge the most important potential confounding factors have been controlled for, which should be enough with regard to the conclusions drawn.

Autoantibody positivity does not give any symptoms and the parents were not informed of their children's autoantibody status. Therefore, it seems unreasonable to assume a reversed causality, i.e. that the parenting stress, divorce or maternal exposure to violence should have been induced by an unknown auto-immunity in the child.

Attrition

The response rates for population-based studies have decreased in Sweden during recent years, and in 1999 Statistics Sweden reported response rates around 70% (Statistics Sweden, 2000). In this light, ABIS had a good response rate (74% completed the at-birth questionnaire) and seems to have captured the population of new parents in southeast Sweden. The sample was representative of Sweden concerning the parents' education and origin (Statistics Sweden, 1999).

Motivating subjects to remain in longitudinal studies is difficult. Even in longitudinal follow-ups of small samples is it problematic to keep a high response rate, often despite repeated personal contacts with the subjects. The fact that the ABIS-project includes 250 well child clinics and set out to follow 17 000 families during five to six years, makes a certain drop-out inevitable. The follow-up rate at 1 year of 67% (completed questionnaires) must be considered acceptable,

but the follow-up rate of 54% (completed questionnaires) at 2.5 years is more problematic. However, there were no differences between the original ABIS-cohort and the follow-up samples concerning: foreign origin, education, and parental age.

Concerning the psychological and psychosocial variables of interest for the current thesis, the parenting stress remained at the same level in the 2.5-years follow-up as at 1 year, and most of the children that had experienced a serious life event during the 1st year of life remained in the study at the 2.5-year follow-up. However, the follow-up rates concerning single mothers at birth, lack of support, and lack of confidence/security were more problematic, ranging from 49-54% at 1 year, down to 34-39% at 2.5 years. We have obviously lost a small, but interesting, risk group in the ABIS-project.

No reminders were used concerning the invitation to the adult attachment interview in order not to irritating or tiring the parents and thus loosing participants, since ABIS already is a very laborious study to participate in. Unfortunately, only 34% of the mothers invited to the interview accepted and were interviewed. However, the distribution of the AAI-categories found (Ds: 19%, F: 77%, E: 4%) was proportionate with the distribution of infant categories found in Sweden (A: 22%, B: 75%, C: 3%) (Van IJzendoorn & Kroonenberg, 1988), which at least partly indicates that the sample was representative.

Future plans within the ABIS-project

There is a need for a more integrated approach, where interaction effects concerning risk and salutary factors are included (i.e. stressors and resources). Inclusion of measures of the child's temperament as well as better assessments of social support and confidence/security are needed, and have been included in the questionnaires at 2.5 (not yet analyzed) and 5.5 years. Social support has been shown to have a positive impact on parenting stress (e.g. Östberg & Hagekull, 2000), and should therefore be included in the analyses of parenting stress. However, the direct positive association between social support and health (Cohen & Ashby Willis, 1985) is probably of less interest concerning young children.

The role of attachment security needs to be further investigated. First, the AAI-study (presented in paper V) could be extended insofar that a new group of children with high risk of developing T1D could be included. In such a case, the interviews already done could be used as controls, since all of the children whose mothers have been interviewed so far had turned autoantibody negative at 2.5 years of age. However, in such a case care must be taken to assure blindness

of the interviewer and the interview coders. Second, attachment has also been assessed with the Relationship Scales Questionnaire (Griffin & Bartholomew, 1994), which is a self-report instrument of 18 questions concerning attachment style. The RSQ was included at 2.5 years.

So far autoimmunity at 1 and 2.5 year has been used as outcome variables. However, better outcome variables are needed and a year from now, data concerning autoimmunity at 5.5 years of age will be available within the ABIS-project. As the children in the project grow older, the number of children diagnosed with manifest T1D will unfortunately also increase, and retrospective examination of trigger mechanisms in prospectively collected data will be possible.

Suggestions for future research

Diabetes is a serious and life long disease and even with good treatment there is a large risk for severe long-term complications. The etiology of diabetes must be understood in order for us to intervene in high risk groups and hopefully prevent some children from falling ill. Prospective longitudinal cohort studies of a multi-disciplinary character are needed to find the trigger mechanisms behind Type 1 diabetes. These kinds of studies are of course very time-consuming and expensive. Still, more scientists hopefully dare start and engage in these types of projects, and not the least that scientific granting agencies are willing to finance and keep financing them although it may take time before there are any results.

To further investigate the stress hypothesis the following issues need to be addressed:

- Which factors cause stress in childhood

- What kind and quantity of stress is harmful, and what might be beneficial

- Effects of prevention and intervention

It would also be important to further study the biological implications of stress in early childhood. However, no suggestions for future studies will be given concerning this issue, due to the psychological focus of the present thesis.

Stress in childhood

There is evidence available suggesting that parental moods, insecure attachment, parenting stress, and experiences of serious life events induce stress in the child

(e.g. Bowlby, 1958; Spangler & Grossmann, 1993; Creasey & Jarvis, 1994; Östberg, 1998). The major question for future studies is how this stress is transmitted. How can even very young infants sense these states in their parents? What subtle signals are being perceived? And why is it so stressful for the child?

This question is closely related to what is called the "transmission gap" within attachment theory, namely that the largest part of the influence of adult mental representation on infant security still is unspecified. Sensitivity and parental behaviours are widely believed to be responsible for the transmission of IWM:s and different studies have tried to reveal evidence. However, the transmission gap was not accounted for by parental sensitivity, as believed, in two separate studies conducted by van IJzendoorn (1995) and Pederson *et al.* (1998). According to these authors, the weak associations were probably due to low reliability in the measurement concerning sensitivity and the fact that sensitivity was measured in the second half of the child's first year. They argued that better measurements and higher inter-rater reliability are needed concerning the assessment of sensitivity. Furthermore, by the second half of the first year the child has already adjusted to the parent's quality of sensitivity making parental lack of sensitivity less obvious and more difficult to observe (van IJzendoorn, 1995; Pederson et al., 1998). Main and Hesse have probably reached the furthest in explaining the transmission gap concerning unresolved/disorganized IWM:s in their careful studies of subtle and slightly unusual maternal behaviour, specifically behaviours that are either frightening to the child or situations where the mothers themselves act frightened (Main & Hesse, 1992).

The transmission of stress from parent to child is obvious, but what are the exact processes involved? Sensitivity and parental behaviours are the most likely candidates.

According to Lazarus and Folkman stress is the discrepancy between demands and available resources (1984). A more integrated picture is needed in the study of stress and its negative impact in early childhood. What constitutes demands and resources in young children? Are a stable temperament, secure attachment patterns, and access to the secure base recourses? Do they constitute salutary factors? Do they in that case have a direct or a buffering effect on child health? And do their opposites constitute risk factors?

The issue of demands and resources in childhood needs to be addressed in order to investigate what kind and amount of stress is harmful, and what might be beneficial, to which individual infant/child. Is chronic stress or repeated short term stress most harmful? The inclusion of biological measures of stress (i.e.

different stress hormones) is also important in the investigation of stress during childhood, in order to reveal the biological pathways of health-related stress effects.

Prevention and intervention

Since it is not possible to investigate the stress hypothesis in controlled experiments, randomized prevention or intervention studies aimed at reducing stress in early childhood might be useful to spread light on long-term health-effects of stress reduction in early childhood. However, there would still be a bit of an ethical dilemma since the general benefits of stress reduction hardly can be questioned.

The current thesis has shown that stress early in life was associated with diabetes-related autoimmunity in young children. It is therefore important to try to reduce negative stress in families with small children. Family intervention programs at the well child clinics, e.g. group discussions for parents aimed at reducing parenting stress could be useful. Discussions of this type should focus on the family situation as a whole (Östberg, 1999) and stress the issue of coping strategies concerning daily hassles, including how to handle poor child sleep. The issue of child sleep and coping strategies for poor child sleep could also be brought up routinely with all new parents visiting the well child clinics.

Despite the fact that no association between attachment insecurity and diabetes-related autoimmunity at 1 year of age was found, further investigation of this topic is needed. Attachment insecurity has earlier proven to have a number of negative effects, including contribution to illness (Maunder & Hunter, 2001) and stress proneness (Nachmias et al., 1996; Luecken, 1998). Prevention studies where parents-to-be were informed and coached about attachment, caregiver sensitivity, and the consequences of insecurity, could perhaps help secure parents to become even more sensitive. Concerning insecure parents-to-be more far-reaching therapy and education would probably be needed to help them changing their internal working models and thereby become sensitive parents.

If studies like the ones suggested above could reduce negative stress in the infants they might also have a secondary positive impact on these children's health. Careful follow-up studies of the children and their health could give valuable hints concerning stress-related outcomes.

The strengths and uniqueness
of the current thesis

- This thesis was based on a prospective longitudinal follow-up of a large population based sample, which was representative of Sweden.

- A stress hypothesis, as part of the beta-cell stress hypothesis closely related to the accelerator hypothesis, was suggested, tested and strengthened.

- Different sources of stress, including psychosocial strain in the family, parenting stress, experiences of serious life events, and attachment security were investigated in association with induction of diabetes-related autoimmunity.

- Maternal responses were un-biased concerning the outcome variable, since diabetes-related autoimmunity (which gives no symptoms), rather than manifest T1D, was used and the parents were not informed of the child's autoantibody status.

- A number of diabetes-related potential confounding factors were controlled for, including diabetes heredity, child infections, exclusive breast-feeding, increased parental age, caesarean section, and need for neonatal intensive care.

GENERAL CONCLUSIONS

- The stress hypothesis – namely that psychological stress, via increased cortisol levels leading to insulin resistance and beta-cell stress, could be involved in the development of Type 1 diabetes – has been strengthened. However, this hypothesis warrants further investigation.

- It is important to reduce negative stress in children and this issue needs priority especially among clinicians working with children. It is central to look at the whole family situation and to try to reduce parenting stress and dissatisfaction with the parenting role, by for example discussing various coping strategies concerning child sleep problems.

- More than 2.5 years follow-up time is needed to investigate the role of psychological stress in the development of Type 1 diabetes. Hence longer prospective cohort studies are required to solve this part of the diabetes puzzle.

- Large-scale screening studies for the prediction of chronic diseases in the general child population can be performed, since participation does not cause increased anxiety at least not in the vast majority of the parents.

SVENSK SAMMANFATTNING

Stresshypotesen

– En tänkbar förklaring till uppkomst av diabetesrelaterad autoimmunitet hos barn?

Bakgrund

Näst efter Finland har Sverige den högsta förekomsten av typ 1 diabetes i världen. Erfarenheter av svåra livshändelser har tidigare i retrospektiva studier visat sig utgöra en riskfaktor för utveckling av sjukdomen, troligen via den biologiska stressreaktionen. Föräldrastress och otrygga anknytningsmönster hos föräldrarna är andra viktiga källor till stress tidigt i barndomen. Psykologisk stress ökar behovet av insulin i kroppen och kan ge upphov till insulinresistans, vilket ökar belastning på de insulinproducerande betacellerna i bukspottkörteln (betacellstress).

Syftet med den här avhandlingen var att föreslå och börja undersöka en stresshypotes – nämligen att psykologisk stress kan ge upphov till insulin-resistans som kan leda till betacellstress, vilket skulle kunna utlösa en auto-immun reaktion mot de insulinproducerande betacellerna i bukspottkörteln hos genetisk predisponerade individer. Om alla betaceller förstörs, t.ex. av den auto-immuna reaktionen mot betacellerna, kan insulin inte längre produceras i kroppen och diagnosen typ 1 diabetes är definitiv.

Metod

Familjer från det prospektiva longitudinella populationsbaserade ABIS-projektet, vilket följer ca 17 000 barn från födelsen till skolåldern, deltog i de empiriska studierna i föreliggande avhandling. Mammorna besvarade frågeformulär, som bland annat innehöll frågor om föräldrastress, svåra livs-händelser, och sociodemografisk bakgrund, vid barnets födelse samt när barnet var 1 respektive 2,5 år gammalt. Otrygga anknytningsmönster hos mamman mättes med hjälp av Anknytningsintervjun. Diabetesrelaterade autoantikroppar, Tyrosine phosphatase (IA-2) och Glutamic Acid Decarboxylase (GAD), fast-ställdes i blodprover tagna på barnen vid 1 och 2,5 års ålder.

Resultat och konklusioner

Föräldrastress och erfarenheter av svåra livshändelser, så som skilsmässa och om mamman utsatts för våld, var kopplat till uppkomsten av diabetesrelaterad autoimmunitet hos barnet, möjligen via insulinresistans och betacellstress. Det

var tre gånger så stor risk att barnet utvecklade diabetesrelaterad autoimmunitet efter svåra livshändelser som skilsmässa eller om mamman varit utsatt för våld. Vi undersökte också ett antal alternativa förklaringsmekanismer, men ingen av dess kunde bortförklara resultaten. Resultaten stöder och stärker den föreslagna stresshypotesen. Stresshypotesen behöver dock undersökas ytterligare innan man kan uttala sig om huruvida psykologisk stress är en riskfaktor för ut-veckling av typ 1 diabetes.

Otrygga anknytningsmönster hos mamman var inte kopplat till uppkomsten av diabetesrelaterad autoimmunitet hos barnet. Dock kan denna brist på koppling bero på metodologiska problem i den studien.

Majoriteten av de föräldrar som deltog i ABIS-projektet kände sig lugnade eller opåverkade av att delta i en stor medicinsk screeningstudie som syftar till att upptäcka barn som löper risk för kroniska sjukdomar. Dessa resultat tyder på att storskalig medicinsk screening kan bedrivas i den allmänna befolkningen utan att skapa ökad oro hos föräldrarna/deltagarna.

APPENDIX 1: Wording of some the measures used

Attitude towards participation

At birth: How do you feel when you know that your child is participating in a laborious study?

much calmer ❐ ❐ ❐ ❐ ❐ much more anxious

At 1 year: How are you affected by participating in this type of laborious follow-up of your child?

much calmer ❐ ❐ ❐ ❐ ❐ ❐ much more anxious

Social support and confidence/security

Do you experience enough support from your social environment for yourself and your newborn baby?   ❐ Yes   ❐ No

Do you feel that you have enough confidence/security so that you can give yourself and your newborn child a good start?   ❐ Yes   ❐ No

Experiences of serious life events

At 1 year: Has the child experienced a serious or dramatic life event (e.g. death, divorce, new caregiver, or something similar)?   ❐ Yes   ❐ No

If yes, what/which? _____

At 2.5 years: Have you experienced anything that you consider a serious life event since your child was born?   ❐ Yes   ❐ No

Structured alternatives to determine specific types of serious life events: divorce, subjected/exposed to violence, serious disease in the family, serious accident in the family, loss of relative, becoming unemployed, spouse/common-law spouse becoming unemployed, and if other, what?_____

The original Swedish wording of some the measures used

Attityd till deltagande

Vid födelsen: Hur känner du dig nu när du vet att ditt barn ingår i en omfattande undersökning?

mycket lugnare ☐ ☐ ☐ ☐ ☐ mycket oroligare

Vid 1 år: Hur påverkas du av att delta i en sådan här omfattande uppföljning av ditt barn?

mycket lugnare ☐ ☐ ☐ ☐ ☐ mycket oroligare

Stöd och trygghet vid födelsen

Upplever du att du har tillräckligt stöd från omgivningen som behövs för dig och ditt nyfödda barn?　　☐ Ja　　　☐ Nej

Upplever du att du har den trygghet och allmänna situation som behövs för att ge dig och ditt nyfödda barn en god start?　　☐ Ja　　　☐ Nej

Svåra livshändelser

Vid 1 år: Har barnet varit med om någon allvarlig eller dramatisk livshändelse (t.ex. dödsfall, skilsmässa, ny vårdnadshavare eller dylikt)?　　☐ Ja　　☐ Nej

Om ja, vad/vilka?_____

Vid 2,5 år: Har Du själv utsatts för något som Du uppfattar som en svår livs-händelse sedan barnet föddes?　　　☐ Ja　　☐ Nej

Strukturerade alternativ vid 1 år: skilsmässa, utsatts för våld, svår sjukdom i familjen, svår olyckshändelse i familjen, anhörig som dött, själv blivit arbetslös, make/a-sambo blivit arbetslös, annat, i så all vad?_____

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