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UNIVERSITY OF TURKU

EARLY CHILDHOOD STRESS REGULATION IN OUT-OF-HOME CHILDCARE AND IN AT-HOME PARENTAL CARE

Associations with child temperament and age – The FinnBrain Birth Cohort Study

Katja Tervahartiala

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"It is good to have an end to journey toward;
but it is the journey that matters, in the end."

– Ernest Hemingway

To my family

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ABSTRACT

Prior research suggests that attendance in out-of-home childcare has many positive effects on child development. Despite the advantages, earlier studies also show that children present with higher cortisol levels in out-of-home childcare day when compared to days spent at home. However, only few studies have followed up the same children longitudinally. Furthermore, earlier studies have not included a comparison group of children, who were cared for at home.

This study aimed at comparing children's diurnal saliva cortisol output in out-of-home childcare and in at-home parental care settings at the child age of 2 and 3.5 years old. Furthermore, the aim was to examine whether child temperament would moderate the associations between the childcare context and diurnal cortisol production. The participants were recruited from the FinnBrain Birth Cohort Study.

The results showed that at the age of 2 years, the children presented with higher cortisol levels in at-home parental care when compared to the out-of-home childcare setting. However, these differences no longer appeared, as the children developed and reached the age of 3.5 years. When investigating the diurnal cortisol profiles, the results suggested higher afternoon cortisol levels in the out-of-home childcare group during the childcare day when compared to days spent at home. This difference appeared both at the age of 2 and 3.5 years old. Furthermore, temperamental surgency was associated with higher total diurnal cortisol production across the childcare settings. However, the association between surgency and diurnal cortisol production diminished along with the child age from 2 to 3.5 years of age.

These findings have implications for understanding the periods of sensitivity in early childhood stress regulation and associations between temperament and cortisol output in different childcare settings.

KEYWORDS: Hypothalamus-pituitary-adrenal axis, saliva cortisol, cortisol awakening response, stress regulation, diurnal cortisol rhythm, early childhood education and care, out-of-home childcare, at-home parental care, temperament, negative affectivity, positive affectivity, effortful control

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TIIVISTELMÄ

Aiemmat tutkimukset osoittavat, että päiväkodin varhaiskasvatus hyödyttää lapsia monin eri tavoin. Siitä huolimatta tutkimuksissa on havaittu, että kodin ulkopuolinen hoitoympäristö voi nostaa lasten kortisolihormonitasoja. Aiheesta ei ole juurikaan pitkittäistutkimusta, eivätkä aiemmat tutkimukset ole huomioineet kotihoidossa olevien lasten vertailuryhmää.

Tämän väitöskirjan tavoitteena oli tutkia varhaislapsuuden stressinsäätelyä sekä kotihoidossa että varhaiskasvatukseen osallistuvilla lapsilla. Lasten kortisolitasoja mitattiin kotona ja päiväkodissa 2- ja 3,5-vuoden iässä. Lisäksi tutkittiin, onko lapsen temperementti yhteydessä lapsen kortisolitasoihin kotona tai päiväkodissa. Lapset valittiin mukaan FinnBrain Syntymäkohorttitutkimuksesta.

Tulokset osoittavat, että 2-vuotiaiden lasten keskimääräiset kortisolitasot olivat korkeammat kotihoidossa kuin varhaiskasvatuksessa. Erot kuitenkin tasoittuivat lasten kasvaessa, eikä niitä havaittu enää 3,5-vuoden kohdalla. Varhaiskasvatukseen osallistuvien lasten kohdalla havaittiin lisäksi, että iltapäivän kortisolitasot olivat korkeammat päiväkodissa kuin kotipäivänä vastaavana ajankohtana. Tämä yhteys ilmeni sekä 2-vuotiaana että 3,5-vuoden iässä. Tutkimus osoitti myös, että kaikkein ulospäinsuuntautuneimmilla (ns. surgency/extroversion) lapsilla keskimääräiset kortisolitasot olivat 2-vuotiaana korkeammat sekä kotona että päiväkodissa. Yhteys kuitenkin heikkeni lasten kasvaessa, eikä sitä havaittu enää 3,5-vuoden kohdalla.

Tutkimus antaa uutta tietoa lapsen stressinsäätelystä erilaisissa hoitoympäristöissä sekä lapsen iän vaikutuksesta kortisolihormonitasoihin. Tutkimus myös lisää ymmärrystä lapsen temperamenttipiirteiden yhteydestä stressinsäätelyyn sekä siihen liittyvistä herkkyysvaiheista.

AVAINSANAT: hypotalamus–aivolisäke–lisämunuaiskuori-akseli, sylkikortisoli, kortisolin heräämisvaste, stressinsäätely, kortisolin vuorokausivaihtelu, varhaiskasvatus, päiväkoti, kotihoito, temperamentti, negatiivinen affektiivisuus, positiivinen affektiivisuus, tahdonalainen itsesäätely

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Abbreviations

HPA	Hypothalamus-Pituitary-Adrenal axis
CRH	Corticotrophin-releasing hormone
AVP	Arginine vasopressin
ACTH	Adrenocorticotropic hormone
GC	Glucocorticoids
CAR	Cortisol awakening response
SES	Socioeconomic status
SED	Socioeconomic disadvantage
ECEC	Early Childhood Education and Care
OECD	The Organization for Economic Cooperation and Development
gwk	gestational week
ECBQ	Early Childhood Behavior Questionnaire
AUC_G	Area under the curve with respect to ground
AUC_I	Area under the curve with respect to increase
AIC	Akaike information criterion
LOESS	Locally estimated scatterplot smoothing
WLS	Weighted least squares regression
nlme	Nonlinear and linear mixed effect models
NA	Negative affectivity
EC	Effortful control
PA	Positive affectivity

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Tervahartiala, K., Karlsson, L., Pelto, J., Kortesluoma, S., Hyttinen, S., Ahtola, A., Junttila, N., Karlsson, H. (2020). Toddlers' diurnal cortisol levels affected by out-of-home, center-based childcare and at-home, guardian-supervised childcare: comparison between different caregiving contexts. *European Child and Adolescent Psychiatry*, 29:1217–1229.

II Tervahartiala, K., Nolvi, S., Kortesluoma, S., Pelto, J., Hyttinen, S., Junttila, N., Ahtola, A., Karlsson, H., Karlsson, L. (2021). Child temperament and total diurnal cortisol in out-of-home center-based child care and in at-Home parental care. *Child Development,* 92 (1): 408–424.

III Tervahartiala, K., Kortesluoma, S., Pelto, J., Ahtola, A., Karlsson, H., Nolvi, S., Karlsson, L. (2021). Children's diurnal cortisol output and temperament in two different childcare settings at 2 and 3.5 years of age. *Submitted*.

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1 Introduction

Out-of-home, center-based childcare constitutes an important environment for many children in Western societies (OECD, 2020). Prior research suggests that Early Childhood Education and Care (ECEC) has positive effects on children's learning, cognitive skills and later academic achievements (Burchinal et al., 2000; Esping-Andersen et al., 2012; Loeb et al., 2007). However, the long-term benefits of the ECEC for educational outcomes are contradictory and often moderated by parental education (Karhula et al., 2017; Wahler et al., 2017). Children enter out-of-home childcare from different socioeconomic backgrounds with a variety of socioemotional competences and different individual characteristics (Suhonen et al., 2016). The ECEC is suggested to alleviate differences, which arise from different family backgrounds, and it may be most beneficial for the children with several risk factors and a disadvantage in their home rearing environment (Berry et al., 2014; Chryssanthopoulou et al., 2005; Eckstein-Madry et al., 2020).

Despite the advantages of the ECEC, earlier studies also suggest that out-of-home childcare is associated with elevated stress levels in some children (Albers et al., 2016; Dettling et al., 1999; Drugli et al., 2017; Groeneveld et al., 2010; Gunnar et al., 2010; Ouellet-Morin et al., 2010; Sumner et al., 2010; Vermeer & van IJzendoorn, 2006; Watamura et al., 2009). Parental separation, a changed caregiving environment and the challenges in peer relations are suggested to be among the factors affecting higher cortisol hormone levels in children participating in out-of-home childcare (Legendre, 2003; Vermeer & Groeneveld, 2017). The development of the child stress regulation systems is suggested to be influenced by the early life environments. The quality of care, parenting practices and a family's socioeconomic status (SES) are among the factors affecting child well-being and regulation capacity (Gunnar & Quevedo, 2007; Gunnar & Cheatham, 2003; Lupien et al., 2000; Pendry & Adam, 2007). Prior research also suggests that a child's individual characteristics, such as temperament, may play an important role in stress regulation and sensitivity to environmental influences (Talge et al., 2008; Turner-Cobb et al., 2008; Watamura et al., 2004).

However, only few studies have followed up the same children longitudinally and examined the associations between childcare contexts and stress regulation at multiple age points. Furthermore, earlier studies have not included a comparison group of children, who were cared for at home. Instead, all studies to date have examined the same children during their out-of-home childcare day and during the day that they spent at home. The current study aimed at filling in this gap by assessing children and their diurnal cortisol excretion both in out-of-home childcare and in at-home parental care settings at the child age of 2 and 3.5 years in Finland. Further, the aim was to investigate the role of temperament as a moderator of child stress regulation in different childcare contexts in a prospective study design.

There are large differences in educational systems and social politics among countries (OECD, 2020). The Finnish ECEC is based on the Nordic model that, originally in the 1970s, aimed at promoting employment and nowadays offers a subjective right for all the children under school age to participate. Currently, the ECEC is also an important part of lifelong learning and focuses increasingly on a child's learning and competences (Karila, 2012; Minedu, 2017). However, the enrolment rate in ECEC is lower, while the at-home parental care rate is higher in Finland than in other Nordic countries. One reason for this may lie in the rather long home care allowance in Finland, which entitles the at-home parental care until the child is 3 years old (OECD, 2020). Also, some Finnish parents consider home care a better option for very young children. At the same time, there are concerns about the children who are cared for at home outside the ECEC as well as their mother's risk for being marginalized from the labor market (Karila, 2012). Also, evidence to date suggests that there are differences in SES between mothers who are returning to work earlier and those who are staying at home longer in Finland (Närvi et al., 2020). Mothers with a higher education and a better employment status usually return to work sooner after the delivery and their children participate in out-of-home childcare instead of being cared for at home (Wahler et al., 2017).

Hence, more longitudinal research is needed to examine how different childcare contexts effect on child stress regulation, which could be considered as one indicator of adaptive development and well-being. More research is also needed to reveal whether a child's individual characteristics, such as temperament, modifies these associations.

2 Review of the Literature

2.1 Perspectives on stress

There are several traditions and perspectives on studying and defining stress. Epidemiological, psychological and biological traditions have reviewed stress from different perspectives but have also integrated during recent decades (Cohen et al., 2016). The epidemiological perspective has focused on individual life events and assumed that specific adverse experiences affect an equivalent amount of stress for all individuals. Estimates are based on the average ratings at the population level. Epidemiological measures have been useful for predicting morbidity, disease progression and mortality (Cohen et al., 2016). Psychological tradition, in turn, emphasizes individual differences in experiences. There are large individual differences in how people respond to a similar stimulus (Lazarus, 1999; Lazarus & Folkman, 1984). The transactional model of stress emphasizes cognitive appraisal in which an individual assesses the demands and available coping resources to manage the challenges. A stressful situation occurs when available resources to cope with demands are inadequate (Lazarus & Folkman, 1987). Stress can also be viewed from a physiological perspective as described in an early work by Selye (1956). The biological tradition of stress has focused on laboratory studies and emphasizes physiological processes in neuroendocrine systems, which are involved in stress reactions. Chronic and repeated stress reactions and an imbalance of homeostasis in the human body may increase the risk for many diseases (Cohen et al., 2016; Jackson, 2012).

Social and psychological challenges in situations with low predictability, low controllability and novelty mostly activate the human stress response system and excretion of the stress hormone cortisol (Kirschbaum & Hellhammer, 1994). Actually, the stress response system of a human is highly sensitive to the social environment. Previous research suggests that natural selection in human evolution has favored the sensitivity to social challenges. That is, social relationships and the ability to cooperate in ever-changing situations have been fundamental to success in our species (Flinn, 2006). Boyce and Ellis (2005) further suggest that the human stress response system has early developmental plasticity, and it has adopted specific features from the postnatal environment to best adapt and respond to the demands in the prevailing early childhood context. Sensitivity to a context has ensured the survival and reproductive fitness for both groups and individuals in human evolution (Boyce & Ellis, 2005). Maternal prenatal stress has shown to be associated with adverse behavioral and cognitive development in offspring (Glover, 2011) as well as over-activation and dysregulation of the child stress regulation system (Gutteling et al., 2005). However, even though increased alertness to danger or aggression to fighting predators and impulsivity have had adaptive value for our ancestors, the stress reactivity caused by less life-threatening stimuli in our modern society may lead to an increased risk for psychopathology (Glover, 2011).

2.2 Stress regulation and reactivity

The brain is a central organ for stress regulation, because it determines what is threatening and also controls behavioral and physiological responses to stress reactions. The brain regions, such as the hippocampus, prefrontal cortex and amygdala, play a role in creating a link between a psychological experience and a physiological stress reaction (McEwen, 2008). Stress regulation refers to an appropriate physiological and psychological response to environmental challenges or threats and the body’s ability to recover in balance and maintain homeostasis (Joseph & Whirledge, 2017). Behavioral facets of the stress response include increased awareness, improved cognition, euphoria and enhanced analgesia to improve performance in challenging situations (Smith & Vale, 2006). There are large individual differences in reactions and responses to stressful situations. Individual reactions may be based on positive and negative experiences in childhood or later in adult life (McEwen, 2008). Previous research also suggests that individual characteristics, such as temperament, play an important role in the sensivity to environmental influences and stress regulation (Phillips et al., 2011).

Physiological effects include the activation of the hypothalamus-pituitary-adrenal (HPA) axis, which is one of the most essential systems in neuroendocrine responses to stress (Figure 1). In a response to a stressful situation, the hypothalamus activates and releases corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP), which pass through small blood vesicles to the pituitary gland and stimulates the release of adrenocorticotropic hormone (ACTH). The ACTH interacts with receptors on the cortex of the adrenal gland and stimulates the production of glucocorticoids (GC), i.e., cortisol in humans, which is the hormonal end product of the HPA axis (Gunnar & Quevedo, 2007; Tsigos & Chrousos, 2002). The HPA axis is also a self-regulating system, as the rising levels of cortisol inhibits further release of CRH and ACTH in an endocrine negative feedback loop, which enables the HPA axis to a return into physiological balance after activation (Guerry & Hastings, 2011; Gunnar & Quevedo, 2007; Joseph & Whirledge, 2017). In addition to the stress regulation, the HPA axis is related to many important functions in the human body. The HPA axis controls and regulates various processes, such as metabolic homeostasis, the cardiovascular system, cell proliferation and survival, growth, cognition and behavior, immune function and reproduction (Joseph & Whirledge, 2017).

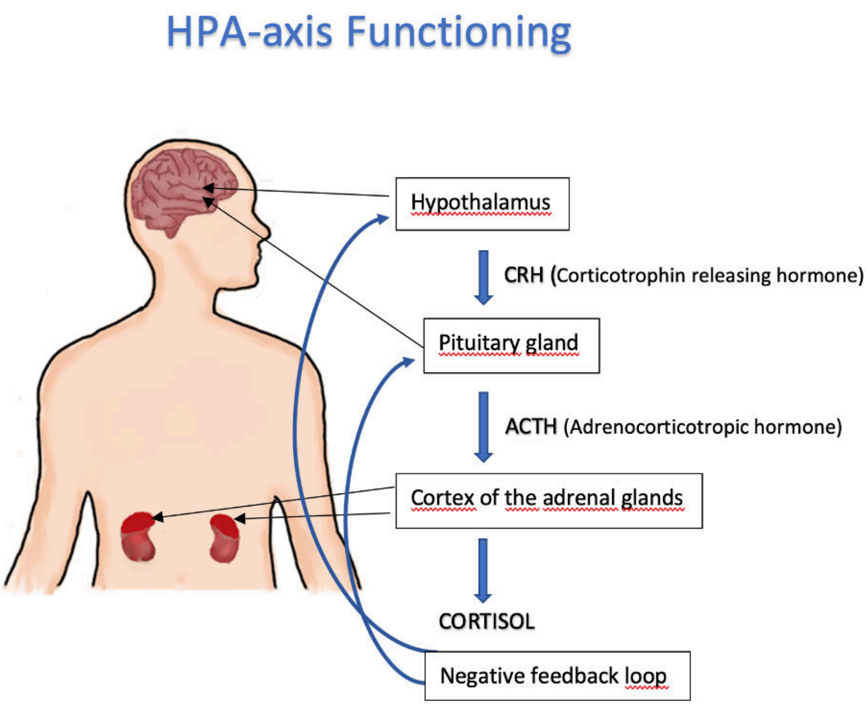

Figure 1. The stress response system.

Physiological stress reactivity is an inevitable part of everyday life and necessary for survival (Gunnar & Quevedo, 2007). Acute stress responses promote adaptation via responses of neural, cardiovascular, autonomic, immune and metabolic systems. However, prolonged and chronic exposure to stress can promote and exacerbate pathophysiology and cause allostatic load. That is, the same systems that regulate stress reactions can cause dysregulation and a disadvantage when exposed to a too high load (McEwen, 2008, 2018). Continuous exposure to high levels of the stress hormone cortisol increases the risk for physical and mental health disorders such as psychiatric disorders, cardiovascular disease, infectious disease and cognitive impairment (Gunnar & Quevedo, 2007; Pechtel & Pizzagalli, 2011). The consequences of stress depend on the type of disadvantage, its frequency and on the timing of the adversity (Pechtel & Pizzagalli, 2011). Chronic cortisol exposure is considered especially harmful during rapid periods of neural developments. That is, the effects of stress strongly depend on the timing in the lifespan, as the brain is particularly sensitive to stress during both early childhood and also at old age, because it undergoes important changes during these periods (Koss & Gunnar, 2018; Luecken & Lemery, 2004; Lupien et al., 2009). The prospective study of Saridjan et al. (2014) indicates that the atypical patterns in diurnal cortisol patterns in early life precedes problems later in life. Infants who showed higher total diurnal cortisol production, a higher cortisol awakening response and flatter cortisol slopes had more internalizing problem behaviors in preschool age (Saridjan et al., 2014). Stress may also have influence on cognitive functions and learning. Mildly elevated cortisol levels may enhance memory functions, but chronic exposure to high cortisol levels is related to memory impairments and a smaller hippocampus volume, which is a brain region related to memory functions (Lupien et al., 2005).

Dysregulation of HPA axis function is among the biological findings behind several mental disorders. However, the mechanisms underlying this dysregulation as well as the potential contribution to behavioral phenotypes remain only partially understood (Baumeister et al., 2014). This dysregulation may appear as chronic hyporeactivity (Fairchild et al., 2018) or hyperactivity of the stress regulation systems or in an inability to return in balance after a stressful situation (Guerry & Hastings, 2011; Nerderhof et al., 2015; Selye, 1956) or in unusual cortisol levels after awakening (Fries et al., 2009; Nerderhof et al., 2015; Pruessner et al., 2003). Current research is ambiguous about whether the dysregulation of the HPA axis is a consequence of stress experiences or alternatively, a pre-existing vulnerability. Evidence to date suggests a U-curve in the dysregulation of the HPA axis towards both hyper- or hyporeactivity appearing as a risk for mental well-being (Baumeister et al., 2014). Some models support the idea that the activity of the HPA axis is changing when exposed to chronic or high levels of stress. The other hypothesis, in turn, supports the view that pre-existing differences in the HPA axis functioning would make some individuals more susceptible to environmental adversities (Guerry & Hastings, 2011).

2.2.1 Diurnal circadian rhythm of cortisol

The HPA axis is activated in a stressful situation, but the daily cortisol production also follows a circadian rhythm in which the cortisol levels are highest after waking up in the morning and decline over the daytime and evening hours (Gunnar & Quevedo, 2007). Figure 2 describes typical diurnal cortisol excretion profiles. In all species, waking-up is a period of a considerable increase in the release of steroid hormones from the adrenal cortex. It is suggested that the increased levels of glucocorticoids at awakening gear the body up and enable essential functions by increasing the amount of available energy (Kalsbeek et al., 2012). Daily alterations in behavior are usually tied to the environmental change in the amount of daylight. However, both in nocturnal and diurnal species, the elevated glucocorticoid excretion is linked to time of arousal (Kalsbeek et al., 2012).

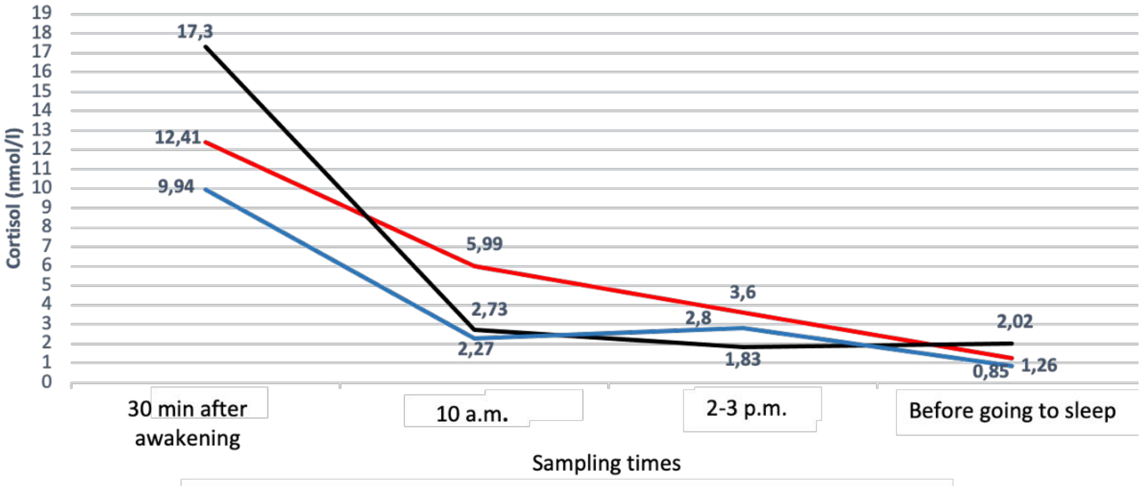

Figure 2. Typical diurnal cortisol excretion profiles.

Infants are born without a circadian rhythm in cortisol, but they normally acquire it during the first year of life. However, there are also large individual differences in stability and the age that children acquire this rhythm (De Weerth et al., 2003). Earlier research suggests that day-to-day stability in total cortisol concentration during a day increases between 30 and 72 months of age. Furthermore, a total amount of cortisol during a day decreases during the early childhood years (Simons et al., 2015). The reason for this may lay in the increased self-regulation capacity along with the child age, which may reduce the conflicts children are involved in and enable them to better regulate emotions and encounter the potentially stressful events (Watamura et al., 2004). Prior research also suggests that there is a hyporesponsive period in cortisol response that begins in infancy and continues later in childhood. Some of the earlier research suggest that during the first year of life, the HPA axis becomes more insensitive to stressors, which protects the rapidly developing brain from the impact of elevated glucocorticoids. However, the exact period of the hyporesponsivity has not been determined (Gunnar & Quevedo, 2007; Lupien et al., 2009).

The cortisol awakening response (CAR) describes the period of increased cortisol production at 30–45 min after awakening (Pruessner et al., 1997). A positive CAR has been observed already in young infants and is maintained consistently throughout the toddler- and childhood years (Bäumler et al., 2013). Most toddlers take naps, which is shown to influence the diurnal cortisol pattern (Tribble et al., 2015). That is, in toddlers, the cortisol production does not only follow a diurnal rhythm but also the patterns of daytime naps. The pronounced cortisol rise is suggested to follow both morning and afternoon naps. However, napping in the morning has resulted most robust post-nap cortisol rise in comparison with the afternoon naps (Tribble et al., 2015).

2.3 Contextual model of child development

The theoretical framework of this study was influenced by the views of Sameroff & Chandler (1975) in which child development takes place in a close and dynamic interaction with the environment. According to the transactional model, behavior and development cannot be separated from the environment such as family, relatives, peers, non-parental care, caregivers and neighborhood. Furthermore, the child is not only passively affected by the environmental influences, but also is an active actor, who influences their environment. In the family context, a child's individual characteristics may affect parenting and a parent's reactions, which will, in turn, have an influence on the child and further to the whole growth environment (Sameroff, 2010).

According to the transactional model, the self is formed from a set of psychological and biological processes, which are interacting with each other. The psychological overlapping aspects include cognitive and emotional realms, mental health, social competence and identity. These psychological domains are subserved and interact with biological processes such as neurophysiology, neuroendocrinology, proteomics, epigenomics and genomics. Together, these aspects comprise the biopsychological self system, which is further interacting with other environmental and social systems such as family, school, neighborhood and community (Sameroff, 2010).

Applying the theory of Sameroff & Chandler (Sameroff, 2009), an out-of-home center-based childcare environment can be viewed in the light of the transactional model. A child's individual characteristics and behavior affect on the group dynamics in an out-of-home childcare setting, and vice versa, the other children and caregivers in the group influence the individual child. Each of these settings are unique and exist in a particular time and in a particular place. The same child could have behaved differently in a different context and also influenced the environment in a distinct way (Sameroff, 2009).

2.4 Early life environments and stress regulation

The development of child stress regulation systems is suggested to be influenced by both prenatal (O'Connor et al., 2013; Van den Bergh et al., 2017) and postnatal environments (Gunnar & Quevedo, 2007). Although, a direct investigation into parental practices or attachment was not conducted in this thesis, it is important to understand how these factors may affect child stress regulation across childhood. Therefore, the following paragraphs will shortly describe these early life environments and their potential role in a child's HPA axis functioning.

2.4.1 Parenting influences on stress regulation

After birth, the early environment and in particular primary caregiving and family practices have a strong effect on the child's HPA axis functioning. Child development takes place in a close relationship with primary caregivers, who play an important role in regulating and supporting a child's emotions and arousals (Gunnar & Quevedo, 2007; Gunnar & Cheatham, 2003). In particular, maternal responsiveness and sensitivity to a child's needs and the quality of interaction have an effect on child stress regulation (Gunnar & Donzella, 2002; Kertes et al., 2009; Tarullo et al., 2017). A secure attachment to the mother also helps children manage in a strange situation and enables adaptation to non-parental care (Ahnert et al., 2004). Sensitive parenting is suggested to form a buffering effect in a child's developing stress regulation system, which enables appropriate cortisol responses to the stress and return to basal levels after challenging situations (Gunnar & Quevedo, 2007; Gunnar & Hostinar, 2015).

Prior research suggests that depressed mothers display more negative parenting, and their children have shown more psychopathology and social withdrawal (Apter-Levi et al., 2016). Maternal depression across the child's first year of life has been associated with a child's disrupted and diminishing HPA axis flexibility later in childhood. Children of depressed mothers have shown a blunted cortisol response during stressful test situations at the age of 6 years (Apter-Levi et al., 2016), which may derive from the children's compromised social-emotional development and failure in the mother's ability to buffer child's HPA axis responses (Apter-Levi et al., 2016; Azak et al., 2013). Earlier results of Feldman et al. (2009), in turn, suggested that maternal depression was associated with higher baseline cortisol and higher cortisol reactivity in infants (Feldman et al., 2009). Similarly, Saridjan et al. (2010) showed that a higher level of parenting stress was related to a higher total diurnal cortisol production in infants indicating that parental symptoms may have a distinct effect on the a child HPA axis functioning in children at different ages.

In addition to maternal depression, maternal anxiety has been associated with a child's cortisol responses in early childhood (Simons et al., 2015). Maternal postnatal anxiety is suggested to play a role in the development of a child's HPA axis functioning and was related to a decreased diurnal cortisol decline in 6-year-old children. The potential underlying mechanisms may lie in the mother's behavior, which is suggested to be lower in sensitivity and lower in responsitivity in mothers with an anxiety disorder (Simons et al., 2015).

2.4.2 Family dynamics and SES on stress regulation

Marital satisfaction of the parents is also among the most important factors affecting child's well-being. An earlier study by Pendry and Adam (2007) suggests that poor marital functioning, i.e., with regular verbal aggression and the seldom use of calm discussion to achieve compromise during disagreements, has been associated with higher average and wakeup cortisol levels both in adolescent and preschool-aged children, when compared to children whose parents reported high marital functioning. Furthermore, poor marital functioning was associated with higher evening cortisol levels and flatter diurnal cortisol slopes in preschool-aged children but not in adolescents indicating some age-dependent variability (Pendry & Adam, 2007). Similarly, disagreements between parents and a child's perceived threats and emotional insecurities were associated with higher cortisol levels (Koss et al., 2013). Additionally, destructive and continuing marital conflicts between parents were associated with flatter cortisol slopes in children, suggesting that chronic marital conflicts may cause a down-regulation of the children's cortisol responses (Koss et al., 2013).

A family's socioeconomic status (SES) as well as socioeconomic disadvantage (SED) are widely associated with child health and well-being (Kim et al., 2018; Letourneau et al., 2013; Vliegenthart et al., 2016). SES is a marker of social position and is usually estimated from the education, the income and the occupational level of the person (Poulain et al., 2020). SED is similar to SES, but it also includes the subjective perception of social position and one of the most commonly used indicators in it is poverty (Kim et al., 2018). There are a variety of mechanisms, which are linked to SES, such as, material resources, social relationships, health behavior, social support and living environment (Bradley & Corwyn, 2002; Poulain et al., 2020). Especially a mother's SES is suggested to associate with a child's health outcomes, and this connection begins already early in life (Lupien et al., 2000; Meyrose et al., 2018). Low family income has been associated with a higher total diurnal cortisol production and a higher cortisol awakening response in infants when compared to controls in high-income families. Maternal smoking during pregnancy, which is supposed to be one indicator of early adversity, was also associated with higher cortisol awakening response in infants (Saridjan et al., 2010). Furthermore, both low SES and a mother's depressive symptoms have been associated with higher cortisol levels in children from 6 to 10 years of age, which may reflect on the influence of early life adversities on the maturation of the HPA axis functioning (Lupien et al., 2000; Saridjan et al., 2013).

In summary, previous research has found associations between caregiving, family influences and child cortisol output. However, the longitudinal effects and enduring associations are less known. The earlier findings are also somewhat contradictory, and the associations seem to depend on moderators such as a child's developmental stage, a family's socioeconomic status, the study design and the stressor type (Hackman et al., 2018). Earlier research has found both increased and decreased diurnal cortisol levels as well as both hyperactive and hypoactive HPA axis functioning in chronically stressed individuals (Wesarg et al., 2020). One possible explanation for hypoactivity may lay in the stress mechanism, which has evolved to downregulate the HPA axis functioning under chronic stress to protect the body from the toxic effects of stress hormones. Thus, chronic stress may first elevate the cortisol levels, but continuous stress may alter the cortisol output below normal secretion. It is also possible that different forms of early life adversities have a distinct effect on HPA axis functioning (Wesarg et al., 2020). More research and comprehensive models are needed to account for pathways related to various adverse conditions as well as age-depended periods of vulnerability. Also, multimethod and multicontext research is needed to consider which neurophysiological systems, such as HPA axis- and immune functioning, are integrated and which factors are moderating these associations (Koss & Gunnar, 2018).

2.5 Stress regulation in the out-of-home childcare context

The majority of children in Western societies participate in center-based, out-of-home childcare (OECD, 2017). Prior research suggests that the high quality of the Early Childhood Education and Care (ECEC) has a positive influence on child language and cognitive skills (Burchinal et al., 2000; Esping-Andersen et al., 2012; Loeb et al., 2007) as well as adulthood sociability and compassion (Gluschkoff et al., 2018; Oksman et al., 2019). The gains of the ECEC are suggested to be especially beneficial for the disadvantaged children and the children from lower socioeconomic families (Larose et al., 2020; van Huizen & Plantenga, 2018). Also, for children having several risk factors and socioeconomic disadvantages in their rearing environment, the out-of-home childcare attendance had a protecting effect, and it was associated with children's decreased cortisol levels and better ability to regulate stress responses (Berry et al., 2014; Chryssanthopoulou et al., 2005; Eckstein-Madry et al., 2020). Even though the high quality of the ECEC is suggested to improve children's language-, reading-, math- or communication skills during their early childhood years (Belsky, Vandell, et al., 2007; Burchinal et al., 2000; Esping-Andersen et al., 2012; Loeb et al., 2007), the long-term benefits of the ECEC for educational outcomes are contradictory and often moderated by maternal education (Karhula et al., 2017; Wahler et al., 2017). Earlier research indicates that children, whose mothers had a higher education, also scored higher in language and cognitive skills later in adolescence regardless of the earlier ECEC participation (Wahler et al., 2017). Prior findings of Saarinen et al. (2019) also indicate that the ECEC participations itself had no effect on learning outcomes, when the children became adolescents. However, participation in the ECEC may enhance a child's adaptation to the classroom and improve learning achievements during their first school years.

Despite the benefits of the ECEC, prior research further suggests that out-of-home childcare and home-based childcare (i.e., non-parental childcare in home environment) are associated with elevated cortisol levels in some children (Albers et al., 2016; Dettling et al., 1999; Gunnar et al., 2010; Vermeer & Groeneveld, 2017; Vermeer & van IJzendoorn, 2006). Higher cortisol levels in non-parental, out-of-home childcare are suggested to be caused by the changed caregiving environment and emotional demands (Vermeer & Groeneveld, 2017). In the out-of-home childcare context, the children need to cope with a parental separation and interaction with multiple adults and peers, which may cause emotional arousal and thus activate the HPA axis differentially as in the at-home parental care (Ahnert et al., 2004; Legendre, 2003; Pihlainen et al., 2020; Vermeer & Groeneveld, 2017). Prior findings also suggest that a child's individual abilities, such as the development of executive functions, are associated with cortisol levels in the out-of-home childcare context. Children presenting with poorer executive functioning had higher cortisol levels between 3 and 5 years of age in out-of-home childcare (Wagner et al., 2016).

The review and meta-analysis of Vermeer and van IJzendoorn (2006) comprised nine studies in which children's cortisol levels in out-of-home childcare were examined. The main finding was that children showed higher cortisol levels in non-parental, out-of-home childcare when compared to days when the same children were at home. The cortisol rise in out-of-home childcare has been mostly observed in children between 24 and 36 months of age when compared to infants or older preschoolers (Watamura, Donzella, Alwin, & Gunnar, 2003). Similarly, a review of Geoffroy et al. (2006) found the lowest cortisol levels in out-of-home childcare in infants aged 3 to 16 months and highest in children aged 39 to 59 months old. These results are well replicated in later studies indicating that the out-of-home childcare attendance is associated with higher cortisol levels in particular from mid-morning to mid-afternoon hours when compared to decreasing cortisol levels at home (Drugli et al., 2017; Groeneveld et al., 2010; Li & Shen, 2008; Ouellet-Morin et al., 2010; Sumner et al., 2010; Watamura et al., 2009). Elevated cortisol levels in out-of-home childcare have also been associated with a lower antibody secretion and more parent-reported illnesses in children being mostly upper respiratory symptoms (Watamura et al., 2010). In most studies, no sex differences were observed, with the exception of the study of Ouellet-Morin et al. (2010), which suggested higher cortisol levels in boys than girls attending out-of-home childcare at the ages of 2 and 3 years.

Total hours per day in out-of-home childcare is one factor that has been associated with cortisol levels. Children participating in full-day and in full-time, out-of-home childcare have presented with higher cortisol levels than children with part-time or half-day enrollment (Lumian et al., 2016). A prior study of Drugli et al. (2017) supports these findings by suggesting that more than eight hours per day in out-of-home childcare was associated with increased afternoon cortisol levels when compared to fewer hours per day in childcare. Hence, long hours in out-of-home childcare may be physiologically demanding, in particular, for very young children.

The quality of care tends to be an important factor affecting child stress regulation in different out-of-home childcare and preschool settings. Prior studies suggest that a low quality of care and conflicts in the caregiver-child relationship are associated with higher cortisol levels both in home-based and in center-based childcare- and preschool settings (Dettling et al., 2000; Groeneveld et al., 2010; Gunnar et al., 2010; Lisonbee et al., 2008; Sajaniemi et al., 2014; Sims et al., 2005, 2006). A higher level of emotional support from teachers, in turn, has been associated with lower cortisol levels during the day in 4-year-old preschoolers (Hatfield et al., 2013). The quality of care could have been determined from the observational data of the caregiver sensitivity and emotional support (Dettling et al., 2000; Groeneveld et al., 2010; Gunnar et al., 2010; Hatfield et al., 2013; Sims et al., 2006) or from the global childcare quality, such as, space, routines, activities and program structure in out-of-home childcare (Groeneveld et al., 2010; Sims et al., 2006).

Furthermore, a secure attachment to caregivers in out-of-home childcare has been associated with decreasing cortisol levels in children during the day. In addition, a secure attachment to the mother creates a buffering effect for the rising cortisol levels in out-of-home childcare especially in high-quality care. Children with a secure attachment have presented with decreasing cortisol pattern during the day (Badanes et al., 2012). However, children with an insecure attachment to the primary caregiver, have shown a flat cortisol pattern both in low- and in high quality of out-of-home care indicating that low maternal attachment may prevent the optimal benefits also in a high-quality childcare environment (Badanes et al., 2012).

However, only few studies have followed up the same children longitudinally and examined whether child age and adaptation to a new childcare environment moderates the associations between the childcare context and stress regulation. The prospective study by Bernard et al. (2015) showed a rising afternoon cortisol pattern in children during a 10-week transition into a new out-of-home childcare setting.

Even though the mid-morning cortisol levels decreased during the adaptation phase to childcare, the mid-afternoon cortisol levels remained higher during the whole 10-week period in out-of-home childcare when compared to days spent at home (Bernard et al., 2015).

A prospective study by Ouellet-Morin et al. (2010) further suggests that 2-year-old children present with a flat diurnal cortisol pattern in out-of-home childcare when compared to their decreasing cortisol levels at home. When examined at the age of 3 years, the same children showed a decreasing pattern of diurnal cortisol both at home and in out-of-home childcare, which is consistent with earlier studies indicating an increasing pattern during the toddler period in out-of-home childcare and a decreasing pattern as the children develop (Vermeer & van IJzendoorn, 2006). However, the study of Ouellet-Morin et al. (2010) further indicated that the overall cortisol levels in children were higher at the age of 3 during the home days when compared to out-of-home childcare days. The authors hypothesized that this may be a signal of change in basal activity, or it may refer to the HPA axis hyperactivity overnight and its down-regulation during the following morning due to the anticipated social demands in out-of-home childcare at that specific age. Also, the children having less experience in childcare presented with higher cortisol levels in out-of-home childcare at the age of 3 years when compared to a home setting. In contrast, the children with a longer experience in childcare showed lower cortisol levels in childcare and a higher cortisol levels at home. Overall, the results suggest that the discrepancy in cortisol patterns between out-of-home childcare and home settings may be transient and be dependent on the child age as well as experience and adaptation into a new caregiving environment (Ouellet-Morin et al., 2010).

In summary, prior studies suggest that children show higher cortisol levels in non-parental, out-of-home childcare when compared to days they are at home. There may be several different factors, such as separation from parents, multiple caregivers and interaction with peers, which may cause emotional and physical arousal in children. It is also suggested that child age plays an important role in stress regulation in a non-parental, out-of-home childcare context. However, there is a notable lack of longitudinal studies in which the same children had been followed from toddlerhood to preschool age. In addition, earlier research has examined the same children during their out-of-home childcare day and during their home day, but the data for the children cared for at home are missing. More research is needed to examine the possible long-term effects of childcare participation on child stress regulation. It is also important to include comparison groups of the children having at-home parental care with no experience of out-of-home childcare.

2.6 Socioemotional competence in early childhood

Children, who were examined in this study, were toddlers, and toddlerhood is an important period for child's socioemotional development. Socioemotional development refers to child social and emotional competence and the ability to interact in social environments (Brownell & Kopp, 2007). The ages between 1 and 3 and the transition from infancy to childhood are significant periods for children. A child's social and emotional world expands due to increased self-awareness, advanced locomotion and communication skills as well as in relationships with peers and caregivers in out-of-home childcare and other environments outside the home (Brownell & Kopp, 2007).

A child's socioemotional competence developes in a close relationship with the rearing environment (Sameroff, 2009). Parenting behavior is one of the most important aspects influencing a child's socioemotional development. Also, an out-of-home caregiving environment becomes more important at that specific age (Denham et al., 2009; OECD, 2020). During early toddlerhood, most children are willing to participate in group play, while they still have their own play alongside mates. However, later in toddlerhood, children begin to share meanings and goals towards a more organized cooperation with peers. Children also initiate to express more social emotions, i.e., guilt, shame or empathy as well as more independent emotion regulation (Denham et al., 2009). Hence, an appropriate and successful cooperation with peers in childhood is a predictor of later mental health and well-being (Denham & Holt, 1993).

Emotional competence refers to the ability to recognize one's own and other's emotions and regulate emotional experiences. Toddlers are able to express all the basic emotions such as happiness, sadness, anger and fear (Rubin et al., 2006). Emotional competence is an important skill for the interaction in relationships with others. Social competence, in turn, refers to the ability to form and maintain developmentally appropriate social relations (Rubin et al., 2006). A socially competent child establishes positive relations with adults and peers, has self-confidence and the ability to collaborate with others (Denham et al., 2009). Social interaction begins already in early infancy, but in toddlerhood, prosocial behaviors and empathy occur (Denham et al., 2009). Temperament characteristics and the factors of social competence are often associated with each other during early childhood. In particular, higher effortful control and a good ability to regulate emotions contribute to better social competence in children (Denham et al., 2003). Altogether, an appropriate socioemotional competence predicts better mental health, less behavior problems and improves resilience to stress (Denham et al., 2003; Denham & Holt, 1993).

2.7 Theorethical perspectives on temperament

Temperament forms a basis for personality development. However, the whole personality of an individual also includes skills, habits, values, defenses, morals and beliefs and develops over the course of life until early adulthood (Rothbart & Bates, 2006). Temperament refers to biologically-based, individual differences in emotional reactivity, activity and self-regulation and is influenced by genes but also to some extent by experiences early in life (Rothbart & Bates, 2006). The core assumptions of the child temperament characteristics vary somewhat across different theories, while they still have same type of classifications (Goldsmith et al., 1987; Zentner & Bates, 2008).

For instance, the behavioral styles approach of Thomas & Chess (1977) focuses on the behavior and interaction between child and the growth environment. Their goodness-of-fit concept emphasizes that a child's healthy development requires parenting that is tailored to a child's individual characteristics (Thomas & Chess, 1989). According to Buss & Plomin (1975), temperament is inherited, relatively stable across childhood and retained into adulthood. They created an EAS model that consists of emotionality, activity, sociability and shyness and emphasizes heredity of the temperament traits and is widely used in genetic studies. Goldsmith and Campos (Goldsmith et al., 1987) defined temperament as individual differences in experiencing and expressing primary emotions and arousals. The temperamental characteristics can be examined by questionnaires but also in laboratory test situations and by observing behavior. The questionnaire of TBAQ includes five scales being activity level, pleasure/positive affect, social fearfulness, anger proneness and interest/persistence. Kagan (1994) in turn appoints behavioral inhibition and approach as central in temperamental dimensions. Longitudinal studies have shown that children, who showed inhibited behavior in infants, also presented with quiet and socially avoidant behavior in unfamiliar situations later in life. Kagan's theory also emphasizes the biological basis of temperament (Kagan, 1994).

In this thesis, the theoretical model developed by Mary Rothbart was utilized. Temperament refers to the constitutionally based individual differences in reactivity and self-regulation (Rothbart, 1981). The reactivity aspect of temperament can be detected from the approach to a novel situation, frustration, the intensity of reactions and the recovery from the responses. The self-regulation aspects of the temperament aims to regulate and modulate that reactivity (Rothbart & Derryberry, 1981). The main assumption is that temperamental differences are based on responsiveness underlying psychobiological processes. According to Rothbart (Rothbart, 1981), temperament consists of three main factors being surgency/extroversion, negative affectivity and effortful control. From infancy onwards, children show considerable variability in their reactions to their environment. Temperament features can be seen in newborns, who show distress and avoidant movements. From 2 to 3 months onwards, approach behavior can be observed from smiling and body movements. Anger and frustration are also observable early in life, while fear appears later by 7 to 10 months (Rothbart, 2007). The Rothbart's model originally assessed infant's temperament during the first year of life, but has later expanded to include toddlers, preschoolers, adolescents and adults (Rothbart & Bates, 2006). The three dimensions of Rothbart's theory correspond widely to the dimensions observed in other temperament theories and are identified across different cultures and socioeconomic settings. Consequently, Rothbart's theory and instruments have become one of the most commonly used theories to measure early childhood temperament.

2.8 Temperament and child stress regulation

2.8.1 Positive affectivity

Child temperament may play an important role in early childhood stress regulation both in out-of-home childcare and also independent of the environment (Albers et al., 2016; Dettling et al., 1999, 2000; Geoffroy et al., 2006; Talge et al., 2008; Watamura et al., 2002, 2003, 2004). Positive affectivity (PA) consists of a child's tendency to high intensity to pleasure. According to Rothbart's theory, the temperament trait of surgency/extroversion includes positive affectivity, a high intensity of pleasure (sensation-seeking) activation level and describes sociability dimensions of temperament (Rothbart & Bates, 2006). Children high in temperamental surgency are considered to be social and seek interaction with peers (Endedijk et al., 2015). However, surgency is also related to approach behavior, which may associate with externalizing behavior and conflicts with peers in some cases (Dollar & Stifter, 2012). Children higher in surgency have also shown larger stress reactivity in experiments with competitive challenge (Donzella, Gunnar, Krueger, & Alwin, 2000). Prior research has also found increases in cortisol production during transition and adaptation into new school systems (Parent et al., 2019). In particular, a higher level of surgency has been related to higher cortisol levels during a transition into a new and unfamiliar school environment (Bruce et al., 2002; Gunnar, 1994; Gunnar et al., 1997; Turner-Cobb et al., 2008). These findings indicate that the associations between temperamental surgency and cortisol excretion may be especially pronounced during the adaptation to a novel environment. It may be that children higher in surgency are physiologically more reactive to environmental stimuli during early childhood and have thus higher basal levels of cortisol. Earlier findings in rodent models support that viewpoint suggesting that active and novelty seeking behavior was associated with higher concentrations of corticosterone hormones (Kabbaj et al., 2000).

2.8.2 Negative affectivity

Negative affectivity (NA) is a temperament dimension that involves negative emotions such as fear, anger-frustration, sadness and discomfort (Rothbart & Bates, 2006). The prospective study by Schmidth et al. (1995) showed that a higher level of NA in infancy and behavioral inhibition in toddlerhood predicted shyness and social wariness during peer play also later in childhood. Behavioral inhibition is sometimes considered its own trait and refers to the behavioral profile of children, who show avoidance and timid behavior towards novel and unfamiliar people, objects or events. Behavioral inhibition is observable already early in infancy (Rothbart & Bates, 2006).

NA and in particular fearfulness have been associated with higher stress reactivity in a laboratory stress test situation in children between 3 and 5 years of age (Talge et al., 2008). Also, 3-year-old children with shyness have shown higher cortisol levels in a strange approach laboratory test situation (Zimmermann & Stansbury, 2004). The longitudinal study by Kagan et al. (1987) in children between 2 and 5 years of age showed that behavioral inhibition associated with higher cortisol levels in an unfamiliar laboratory test situation. Hence, fearfulness and behavioral inhibition are the dimensions of temperament that correlate with cortisol increases in particular to strange and novel situations (Gunnar & Donzella, 2002).

Furthermore, a child’s NA characteristics have been associated with cortisol production in an out-of-home childcare context (Geoffroy et al., 2006; Phillips et al., 2011). NA and specifically social fearfulness were associated with higher cortisol levels in infants participating in out-of-home childcare (Albers et al., 2016; Watamura et al., 2003). For older preschoolers, NA and shyness have been associated with higher cortisol levels over the day in out-of-home childcare and preschool (Dettling et al., 1999, 2000; Watamura et al., 2002). Childcare quality may also affect differentially children with different individual characteristics. For instance, more inhibited children have shown a lower level of anxiety and vigilance and fewer internalizing symptoms in a higher quality of care, where their cortisol levels were lower when compared to lower quality settings with frequently higher cortisol levels (Gunnar et al., 2011).

A prior study of Gunnar et al. (2010) further suggests that angry and aggressive behavior in boys and anxious and vigilant behavior in girls were associated with larger cortisol increases in non-parental, home-based childcare in children between 3 and 4.5 years of age. The research of de Haan et al. (1998) supports this conclusion by suggesting that higher cortisol levels associate with assertive, angry and aggressive behavior in children when starting preschool and entering a new social situation. However, home cortisol levels were correlated with a more shy, anxious and internalizing behavior. Thus, child temperament may be differentially associated with cortisol production in familiar and novel contexts (de Haan et al., 1998).

2.8.3 Effortful control

Effortful control (EC) describes top-down, self-regulatory aspects of temperament, such as the child's ability in emotion regulation, inhibitory control, attentional focusing, persistence and ability to perform in an appropriate way under conflicts (Rothbart, 2007; Rothbart & Bates, 2006). EC develops during the childhood years and is suggested to be a strong indicator of later academic achievement and for a lower risk for psychopathology (Bridgett et al., 2015; De Pauw & Mervielde, 2010). In turn, a weak ability to regulate oneself in childhood has been associated with behavioral problems (Eisenberg et al., 2005). Strong EC skills are important in social relations, and children higher in EC have shown better social skills and more interaction during peer play (Endedijk et al., 2015). Recent findings further indicate that maternal behavior influence the development of a child's EC. Unpredictability in maternal signals has been associated with lower EC and poorer executive functions in children (Davis et al., 2019).

There are also several studies reporting associations between EC and child stress regulation. Evidence to date suggests that toddlers with less mature EC have presented with higher total diurnal cortisol production in their daily activities (Watamura et al., 2004). A lower level of effortful control has also been related to higher cortisol levels during the transition into a new and unfamiliar school environment (Hall & Lindorff, 2017). In a controlled laboratory test situation, a lower EC was associated with higher stress reactivity in a frustration-eliciting task. In the fear-eliciting task, a lower EC was associated with a flatter cortisol slope indicating that different dimensions of EC, such as emotion regulation and attention, differently interact in a stressful context (Mayer et al., 2014). The family SES may also moderate the associations between EC and cortisol production. A low EC was related to HPA axis dysregulation in children in a community sample of low-income families. The specific mechanisms behind this dysregulation was not clear. The authors hypothesize that a flat slope and low cortisol levels in those children may reflect the downregulation of the HPA axis functioning with a potentially chronic stress, which is also involved in the development of child regulatory functions (Lengua et al., 2013).

Prior research also suggests that the combination of reactivity and regulatory capacity is important in child stress regulation. For instance, Gunnar et al. (2003) reported that the combination of high surgency and poor effortful control was associated with higher cortisol levels in preschoolers through a pathway mediated by an aggressive interaction with peers and peer rejection. This may indicate that some children high in surgency have a higher tendency for conflicts with their peers (Dollar & Stifter, 2012). That would be especially pronounced in children with a lower capacity to regulate emotions. Also, impulsivity, aggression and poor self-control have been associated with higher overall cortisol levels in an out-of-home childcare context (Dettling et al., 1999). Thus, a good ability to control emotions may play an important role in predicting child stress regulation in childcare contexts. In a study of non-parental home-based childcare, the children with more negative emotionality and poorer regulatory competence showed increased cortisol levels in lower quality care. These children would have probably needed most the supportive caregiving to help them to regulate emotions (Dettling et al., 2000).

2.8.4 Individual differences in environmental sensitivity

One mechanism explaining the relations between temperament and childcare attendance could be the individual differences in environmental sensitivity in children with different temperament phenotypes (Belsky, Bakermans-Kranenburg, et al., 2007; Pluess, 2015). An increasing body of research on differential susceptibility initiated by Belsky (2005) suggests that children are, for temperamental and genetic reasons, differentially susceptible to environmental influences. Especially, the temperament trait of NA may be one marker of susceptibility to both adverse effects of unsupportive parenting and, on the other hand, of the beneficial effect of supportive rearing in early life (Belsky, 2005; Groeneveld et al., 2012; Slagt et al., 2016). A prior study by Pluess and Belsky (2009) focused specifically on the out-of-home childcare context in testing differential susceptibility. Children with higher NA exhibited more behavioral problems than controls in low-quality care, but fewer behavior problems than controls in high-quality care. That is, children with more NA benefited more from high-quality care than children with less NA. Thus, the children with higher NA were more affected – for better and for worse – by the childcare quality (Pluess & Belsky, 2009). Also, the study by Phillips et al. (2012) suggested that a high quality of care matters more for some children depending on their temperament characteristics. Children higher with either positive or negative reactivity were more sensitive to the quality of care in out-of-home childcare when compared to children with more average temperamental reactivity (Phillips et al., 2012).

The associations between the environment and a child's individual characteristics could also be explained by the diathesis-stress/dual risk theory, where the vulnerable individuals show more negative outcomes, when they are exposed to an adverse environment in comparison with the more resilient individuals. However, no differences between the groups emerge in a positive environment, as in the differential susceptibility theory in which the vulnerable individuals benefited more from the positive environment (Pluess & Belsky, 2010).

Another approach linking temperament and stress regulation in the out-of-home childcare setting is based on the idea that the temperament may determine the individual fit with the characteristics of the care, such as the childcare practices and caregiver support. There is a goodness-of-fit, when the child's temperament and behavioral style are in balance with the demands and expectations of their environment. On the other hand, there is a poorness-of-fit if the child cannot cope with the environmental demands because of the individual temperament traits (Thomas & Chess, 1989). A child's high NA has been considered especially challenging for parents and caregivers, and it may have different effect on well-being depending on the way the environment reacts and supports the behavior. The research by De Schipper et al. (2004) supports this idea suggesting that daily stability in out-of-home childcare affects differently on children with a difficult temperament than for more easy-going children. That is, adverse effects in childcare were most prominent for more difficult children disturbing the adjustment and the adaptation to the childcare.

2.9 Out-of-home childcare and preschool concepts

There are large differences in educational systems and social politics among countries. The age at which children start in the Early Childhood Education and Care (ECEC) varies among countries. The ECEC is usually closely linked to an individual country's social policy and should be viewed against politics, which guides a family's decision-making and their possibilities to receive services. One perspective to examine differences between countries lays on the social political theories of different welfare regimes. Esping-Andersen (1990) determined the different welfare state regimes as being conservative, liberal and democratic (Esping-Andersen, 1990). In the liberal states such as the United States, the United Kingdom, Australia, Canada and New Zealand, the welfare services are mostly based on the private insurances, and public services are primarily intended for the low-income families. In the conservative models, i.e., in France, Italy and Germany, the social security is connected to employment. The Nordic countries are described as the democratic countries in which the education, social support and health services are universal and provided by the public sector and financed by the tax revenue (Esping-Andersen, 1990). However, there are differences among countries within regimes, and the principles may change over time (van der Veen & van der Brug, 2013).

The ECEC is defined as a systematic education and care for children from birth to primary school age. The structure of ECEC varies among countries, because the beginning of the preschool varies between 3 and 6 years of age (Minedu, 2017; OECD, 2020). There are also large differences in characteristics, the types of services available and hours per day children can attend on the ECEC in different countries. The OECD worldwide categorization of the ECEC systems is defined as follows:

1) center-based ECEC for children under the age of 3, which may have educational functions but is mostly emphasized on care and is usually full-time participation;
2) center-based ECEC for children from the age of 3, which is often integrated to educational system and is called preschool or kindergarten in many countries;
3) family-based or home-based ECEC, which is mostly for children under the age of 3 years and may have educational functions and is often part of the regular ECEC (OECD, 2020). Family-based childcare or home-based childcare is non-parental care and normally organized into small groups in a caregiver's or child's own home or in another home-like environment (Minedu, 2017).

The enrolment rate to ECEC in children ages 0 to 2 years varies enormously across the countries. On average, in the OECD countries, about 35% of the children aged 0 to 2 years participate in out-of-home childcare and gain the ECEC. However, there are large differences among countries, and the enrolment rate tends to be highest, about 50%, in many of the Nordic countries except in Finland, where it is only 31.2%. The participation rate to ECEC for 3- to 5-year-old children is higher than the younger children across countries. In some countries, this is the age of the beginning of compulsory preschool. The majority, 87.2%, of the children between 3 to 5 years of age attend the ECEC in out-of-home childcare centers or in primary education, i.e., preschools or kindergartens in the OECD countries. However, there is again variation among countries as in many European countries, e.g., in France, UK, Belgium, Ireland, the enrolment rate is nearly universal except in Switzerland, where it is only 49.5%. Also, in the Nordic countries, the participation rate is over 90%, except in Finland, where it is 79.5%. However, the enrolment rate increases along with the child age, as at the age of 5 years, the OECD average is 95.4%, and also in Finland, the enrolment rate is already 85% (OECD, 2019).

Table 1. Enrolment rates in registered out-of-home childcare programs and the length of home care leave in weeks in the Nordic countries and in part of the OECD countries (OECD, 2019).

	0–2 years (%)	3–5 years (%)	Maternal and home care leaves in weeks[1]
Iceland	59.7	97.4	26
Norway	56.3	96.9	91
Denmark	55.4	97.5	50
Sweden	46.6	94.1	55.7
Finland	**31.2**	**79.5**	**161**
OECD average	**35.0**	**87.2**	**53.9**
EU average	**32.7**	**88.8**	**65.8**
Luxembourg	63.3	87.5	37.3
Netherlands	59.3	94.5	16
Belgium	56.1	98.4	32.3
Switzerland	38.0	49.5	14
France	56.3	100.0	42
Germany	37.2	94.6	58
United Kingdom	37.7	100.0	39
Portugal	36.7	90.9	30.1
Spain	36.4	97.1	16
Italy	29.7	93.9	47.7
Austria	21.0	89.3	60
Ireland	32.1	98.4	26
Hungary	16.3	92.0	160
Poland	9.5	81.6	24.5
United States	28.0	66.1	0
New Zealand	50.1	94.6	18
Australia	39.6	84.0	18
Estonia	29.1	91.1	166
Slovak Republic	1.3	74.9	164

[1] Total duration of paid maternal leave and home-care leave (length in weeks).

The reason for the differences in the enrolment of ECEC may lie in the parental leave and family support systems. In particular, the home-care allowances have large differences among countries. Parental- and home-care leaves are typically employment-protected leave for the absence of the work that allows the parent to stay home until the child is between 1 and 3 years old. The payment rates and the lengths of the parental leaves vary between countries. In the OECD countries, the mothers are allowed on average 18 weeks paid maternity leave after the child's birth, except in the United States, which entitles no paid leaves for parents. The majority of the OECD countries offer the payment, which covers about 50% of the earlier earnings. The maternity-leave period is followed by parental or home-care leave, which varies considerably across countries. Some of the countries have no home-care leave at all, and some countries, i.e., Finland, Estonia, Hungary and the Slovak Republic, allow the leave until the child is 3 years old. The OECD average for home -care leave is 35 weeks. However, the parental- and home-care leave payments are lower than during the maternity-leave period, and the lowest payments are often in countries with the longest entitlements (OECD, 2019).

Also, the fees of the out-of-home childcare attendance differ considerably across countries. The average cost for two children aged 2 and 3 years old in full-time care is about 27% of the average cross earnings of the family in the OECD countries. However, the amount varies from as low as 3.1% in Austria to 70% in Switzerland. In Finland, the fee is on average 15% of the gross earnings. Center-based childcare is most expensive in countries such as Australia, New Zealand, the United Kingdom, Netherlands, Luxembourg, the Slovak Republic and Switzerland, where the childcare fees are over 50% of the gross earnings. However, these OECD comparisons do not consider the governments' financial support, for example, to single-parent families or families on low income. Some countries may also admit tax benefits and other allowances, which decrease the total costs of the childcare fees (OECD, 2019).

2.9.1 Early childhood education policy in Finland

The ECEC system in Finland is based on the Nordic model that aims to promote democracy for Nordic citizens. Already in the 1970's, women's participation in the labor market presumed a care for children, and the ECEC became an integral part of family policy. At present, the ECEC is also an important part of lifelong learning and focuses increasingly in child's learning and competences (Karila, 2012).

In Finland, all children, who have not reached school age, have a subjective right to the ECEC regardless of parent's employment status (Minedu, 2017). Out-of-home childcare is highly regulated and structured, and legislation determines the group sizes in childcare centers and personnels' education qualities both in public and private childcare units (Minedu, 2017). The quality of ECEC has been assessed very high both by parents and childcare teachers (Hujala et al., 2012). Also, the children are considered as active participants, and their voice has been emphasized when evaluating the quality of Finnish ECEC. In general, children between 2 and 6 years of age have described their ECEC experiences in a very positive way, and they have shared more positive than negative perspectives. Most of the negative experiences concerned conflicts in peer relations or a dislike in some other daily activities

(Pihlainen et al., 2020). All the caregivers in childcare centers need to have an appropriate education for working with children. Two-thirds of the personnel must have a tertiary degree in education, and other personnel must have at least secondary level education (Närvi et al., 2020). Childcare teachers must have at least Bachelor's level degree in Education or in Social Services. The Ministry of Education and Culture is responsible for the ECEC as well as an obligatory pre-primary education that starts at the child age of 6 years. The Ministry issues a national core curriculum on the basis of which municipalities and childcare implement the local curriculum. Furthermore, a personal ECEC plan is drawn up for each child to determine individual development and learning goals and special support when needed (Minedu, 2017).

The childcare fees are determined based on the family's income level and the number of hours that a child participates in early childhood education and care. The maximum fee, in 2020, for the first child in childcare was EUR 289 per month and the minimum EUR 27. The fees were lower for the next children in the same family participating in childcare. In addition, the childcare attendance for the lowest income families was free of charge. The pre-primary education in Finland is also free of charge (Minedu, 2017). Home-care allowance is very popular in Finland, and most families use it at least for some months after parental and paternity leave (Närvi et al., 2020). Children in immigrant families are cared for at home longer than Finnish children. However, their older siblings are more often in the ECEC, whether the mother is staying at home with younger children (Närvi et al., 2020).

There is also large socioeconomic variation between mothers who are returning to work earlier or staying at home for a longer period. Earlier findings indicate that mothers of 2-year-old children were more likely to be working based on whether they already were employed before the child was born and whether they had higher incomes and a university-level education. Therefore, their children also participated in the ECEC from a younger age. Correspondingly, mothers, who had not been employed before the child was born or had not found a job during the home-care allowance, most probably cared for their child at home and used home-care allowance for a longer period. They also had a lower education and a lower level of income (Närvi et al., 2020). Hence, there have been some public concerns about these children who are cared for at home outside the public ECEC and their mother's risk for being marginalized from the labor market (Karila, 2012).

2.10 Summary of the current literature

Child development happens in a close and dynamic interaction with the rearing environment. The child is not only affected by the environment but is also an active actor who influences the environment. A child's social and emotional world expands, and socioemotional competence develops during the toddler period due to increased self-awareness and relationships with peers and caregivers in out-of-home childcare and other environments outside home. Having a good ability to regulate emotions and developed social competence is also suggested to improve child stress regulation skills.

Stress is a normal part of everyday life, but chronic exposure to high cortisol levels may have adverse influences especially for young children. Chronic cortisol exposure is especially harmful during rapid periods of brain development in early childhood. There are several different factors affecting the development of a child's stress regulation systems. For instance, sensitive parenting and maternal responsiveness protect a child's developing stress regulation systems. Maternal depression or anxiety, in turn, is suggested to be associated with less sensitive parenting and thus adverse child outcomes. Also, a family's SES and parent's marital satisfaction are among the factors affecting child development and well-being. Poor marital functioning may affect the whole atmosphere at home. A low SES, which may be linked to lower material resources, weaker social relationships and worse health behavior, may also be risk for adverse child development.

Besides parental care, non-parental, out-of-home childcare forms an important environment for many children in western societies. A high quality of ECEC is suggested to have many positive influences on child development, and it may alleviate the differences which arise from the family backgrounds. However, alongside with the advantages, prior research suggests that non-parental, out-of-home childcare is associated with elevated cortisol levels in some children. In the out-of-home childcare context, the children need to cope with a different caregiving environment, parental separation and interaction with multiple adults and peers, which may cause emotional arousal and thus affect HPA axis functioning. The cortisol rise in out-of-home childcare is mostly observed amongst youngest children and during the mid-afternoon hours.

Prior research also suggests that child temperament may play an important role in early life stress regulation and sensitivity to environmental influences. Child temperament is also suggested to be among the factors associating with cortisol levels in out-of-home childcare. In particular, children with higher negative affectivity and social fearfulness have presented with higher cortisol levels in the out-of-home childcare context. Also, older children with shyness, impulsivity and aggression have shown elevated cortisol levels in out-of-home childcare and in preschool. However, the results are somewhat contradictory, and there is a notable lack of longitudinal research following up the same children across the early childhood years. There are also large differences in childcare concepts and early educational systems as well as political guidance between countries, and the results may not be fully comparable with each other. In addition, earlier research has not included a comparison group of children, who were cared for at home with no experience of out-of-home childcare. Prior research has mainly examined the same children during their out-of-home childcare day and a day spent at home.

The major aim of this dissertation was to examine the same children in a prospective design both in out-of-home childcare and in at-home parental care settings. The FinnBrain Birth Cohort study enabled a unique multidisciplinary context and the opportunity to combine biological samples with birth cohort questionnaires and national registry data.

3 Aims

The aim of the current study was to compare child stress regulation in out-of-home, center-based childcare and in at-home parental care settings at a child's age of 2 and 3.5 years old. The specific goals were to examine total diurnal cortisol production and diurnal cortisol levels in two different childcare settings and at two different measurement days, i.e., Sunday and Monday. Furthermore, an aim was to examine whether child temperament is associated with total diurnal cortisol production, and whether the association between childcare context and diurnal cortisol output is moderated by a child's temperament characteristics. The specific goals of these thesis were:

1. To compare children's diurnal saliva cortisol levels in out-of-home, center-based childcare and in at-home parental care settings at the age of 2 years. Furthermore, to compare diurnal saliva cortisol levels between measurement days, i.e., Sunday and Monday, within childcare settings (Study I).
2. To examine whether child temperament is associated with total diurnal cortisol production in out-of-home, center-based childcare or in at-home parental care at the age of 2 years. Furthermore, to examine whether the association between childcare context and total diurnal cortisol production is moderated by child temperament characteristics (Study II).
3. To compare total diurnal cortisol production between children in out-of-home childcare and children in at-home parental care at the child age of 3.5 years. Moreover, to compare both total diurnal cortisol production and afternoon cortisol levels between the measurement days within both childcare groups. Furthermore, the aim was to examine whether child temperament would be associated with total diurnal cortisol production in the whole study population at 3.5 years of age. The final goal was to investigate whether the associations among a childcare group, child temperament and diurnal cortisol retain or change as the children develop from 2 to 3.5 years of age (Study III).

4 Materials and Methods

4.1 Participants and study design

The participants were drawn from the larger FinnBrain Birth Cohort Study (n = 3808), which is a population-based, pregnancy cohort with aims to identify biomarkers related to prenatal stress and early life stress exposure as well as trajectories for common psychiatric and somatic illnesses (www.finnbrain.fi). Recruitment took place during the first ultrasound visit during gestational week (gwk) 12 in the city of Turku in Southwest Finland, municipalities in the Turku area and the Åland Islands. According to the study inclusion criteria, the nurses approached families with a sufficient knowledge of Finnish or Swedish and selected children with a normal fetal ultrasound screening result. The FinnBrain Research is multidisciplinary with several national and international collaborators and researchers from many different fields. The follow-up of the children will continue for several years (Karlsson et al., 2018).

Research recruitment for the present sub-study was carried out through personal contact by the research personnel between April 2014 and July 2017. When the current study began, more than half of the FinnBrain Birth Cohort children had reached the age of 2 years and were no longer eligible to participate. Hence, a total of 1881 families, whose child was at an appropriate age, were approached by e-mail and provided preliminary information about the childcare sub-study. Altogether 616 families either responded to the e-mail, or they were personally contacted by the research team in order to assess their eligibility and interest to participate in the study. Out of those who had been contacted, 79 refused to participate, and 318 did not meet the inclusion criteria of the current study. Children, who attended either out-of-home, center-based childcare or were cared for at home, were eligible to the study. Children attending other forms of childcare (e.g., family-based childcare, which is childcare operated in small groups in the caregiver's own home; or 24-hour center-based, out-of-home childcare services) were excluded from this study. Family-based childcare was excluded, because the context and group setting are different than in center-based, out-of-home childcare. The 24-hour childcare service was excluded, because the daily hours spent in childcare varied, and it would have been difficult to follow the study protocol. Part-time childcare was also excluded, if the child attended childcare only few hours per day or few days per week. Some of the children were just in transition from parental home care to out-of-home childcare at that specific age and were thus not recruited. The child was also not eligible to participate in the study if the family had moved, and no longer lived in the research area. Finally, a total of 219 children were eligible and recruited to the study. The recruitment process is illustrated in Figure 3.

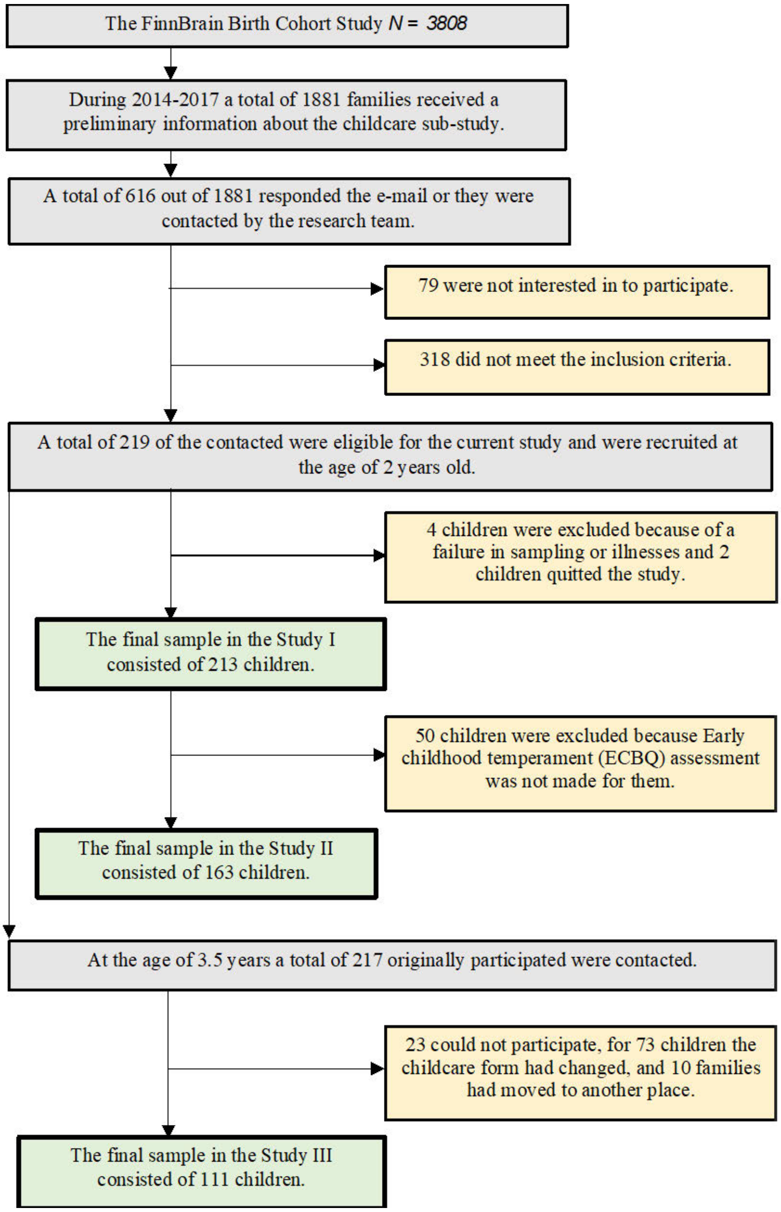

Figure 3. Flowchart of the recruitment process and participants in Studies I–III.

Altogether 219 children participated in Study I. Ultimately, 2 children quit during the early stage, and 4 children were excluded because of failure in saliva sampling or ongoing medication or illness that possibly affected cortisol values. One of the excluded children had extremely high cortisol values. However, the mother had not reported any specific medication, disease or failure in sampling, but those samples were removed as outliers in our statistical analyses. We assumed that samples were contaminated or had some other sources of unknown inaccuracy affecting the results that could not be controlled for in this study. There were also three children, who had one extremely high cortisol value during the collection days. Four children had one single sample that was taken in a wrong place, and thus, did not follow the study protocol. These samples were removed, and the rest of the samples were retained in our analyses.

The final sample in Study I consisted of 213 children of which 106 were attending out-of-home, center-based childcare, and 107 were cared for at home. The mean age of the children in the whole sample was 2.1 years (SD 0.6). The mean age of the children was higher in the out-of-home childcare group being 2.3 years (SD 0.6), while in the at-home parental care group, the mean age of the children was 2.0 years (SD 0.5). The proportion of boys and girls was in balance between the groups. In the out-of-home childcare group, the number of boys was 63 and girls 43, while in the at-home parental care group, the number of boys was 53 and for girls was 54 (Table 5).

The main caregivers in the at-home parental care group during the study participation were mothers (n = 91), and in those being a minority, fathers (n = 10) relatives (n = 4) or other caregivers at home (n = 2). More than half (57%) of the children in the at-home parental care group had siblings at home concurrently, and the number of them varied from one (41.1%), two (11.2%) or three (4.7%) siblings at home during the study days. The child-to-caregiver ratio in the at-home parental care was 1.78 (SD 0.8). Most children at that age take daytime naps. 72% (n = 77) of the children were reported to take naps on Sunday, and 71% (n = 76) reported naps on Monday.

Most children in the out-of-home childcare group had attended childcare already for months and were no longer beginners. A total of 74.5% of the participants had attended more than 3 months in childcare. The duration of childcare attendance varied from less than 2 weeks to 2 years with a mean of 9.6 months (SD 7.3). The children were divided across 32 different childcare centers of which 14 were private and 18 were public childcare units. Furthermore, the children were spread across different groups within the childcare centers, and thus, not clustered in particular centers. Most children (n = 76) participated in full-time childcare, and only a small number (n = 30) had part-time childcare. Full-time childcare included on average 20 childcare days within a month, and a part-time childcare consisted of maximum 16 days (n = 24) or 11 days (n = 6) within a month. The average group size in the childcare centers was 13.47 (SD 3.8) children, and the child-to-caregiver ratio was on average 4.59 (SD 1.2). Children participating in out-of-home childcare followed an equal ECEC program defined by the Finnish government. The daily rhythm was rather similar across the childcare centers. Most children had daytime naps, and a total of 62.3% (n = 66) reported taking naps on Sunday, and 88.7% (n = 94) reported naps on Monday during the out-of-home childcare day. A more detailed daily schedule and cortisol values are reported in Table 2 from Original Publication I.

Table 2. Descriptive statistics of saliva sampling and cortisol (nmol/l) values in Study I.

		Sampling time	**Time between wake-up and sampling (in minutes)**		**Raw cortisol values (nmol/l)**
	***n*[1]**	***M (SD)***	***M (SD)***	***n*[3]**	***Median (Interquartile range)***
Out-of-home childcare group (*n = 106*)					
Child woke up; day 1	97	07:37 (0:51)			
Sampling after waking up; day 1	95	08:04 (0:50)	27 (27)	93	9.71 (5.61–13.14)
Child had meal; day 1	93	08:59 (1:23)			
Sampling at 10 a.m.; day 1	97	10:10 (0:26)		96	3.00 (2.22–4.81)
Child had meal; day 1	98	12:55 (1:16)			
Child woke up from the nap; day 1	66	14:20 (0:57)			
Sampling at 14–15 p.m.; day 1	98	14:46 (0:45)	37 (44)[2]	96	2.69 (1.87–4.17)
Child had meal; day 1	97	19:22 (1:04)			
Sampling before sleep; day 1	95	20:10 (0:54)		93	1.01 (0.67–1.98)
Child fell asleep; day 1	96	20:52 (0:57)			
Child woke up; day 2	101	07:01 (0:32)			
Sampling after waking up; day 2	100	07:14 (0:35)	14 (18)	97	8.76 (5.87–12.84)
Child arrived at child care; day 2	86	08:06 (0:39)			
Child had meal; day 2	80	08:15 (0:27)			
Sampling at 10 a.m.; day 2	96	10:05 (0:17)		99	3.04 (2.28–4.11)
Child had meal; day 2	95	12:02 (1:13)			
Child woke up from the nap; day 2	94	13:47 (0:15)			
Sampling at 14–15 p.m.; day 2	94	14:15 (0:26)	27 (29)[2]	91	4.15 (2.43–6.98)
Child had meal; day 2	96	19:25 (0:58)			
Sampling before sleep; day 2	95	20:11 (0:48)		97	1.04 (0.70–1.69)
Child fell asleep; day 2	97	20:57 (0:50)			
At-home childcare group (*n =107*)					
Child woke up; day 1	104	07:58 (1:10)			
Sampling after waking up; day 1	103	08:28 (1:06)	30 (34)	100	10.65 (6.22–18.61)
Child had meal; day 1	91	09:17 (1:31)			
Sampling at 10 a.m.; day 1	97	10:25 (0:47)		97	4.65 (3.02–7.02)
Child had meal; day 1	102	12:57 (1:28)			
Child woke up from the nap; day 1	77	14:27 (1:13)			
Sampling at 2–3 p.m.; day 1	103	14:59 (0:55)	37 (42)[2]	98	4.27 (2.39–7.53)
Child had meal; day 1	99	19:27 (1:11)			
Sampling before sleep; day 1	101	20:22 (0:51)		97	1.38 (0.76 - 3.52)
Child fell asleep; day 1	101	20:53 (2:00)			
Child woke up; day 2	105	07:43 (0:56)			
Sampling after waking up; day 2	103	08:02 (0:54)	20 (17)	96	11.16 (7.60–16.52)
Child had meal; day 2	100	08:55 (1:13)			
Sampling at 10 a.m.; day 2	104	10:25 (1:01)		103	4.03 (2.70–6.20)
Child had meal; day 2	100	12:54 (1:24)			
Child woke up from the nap; day 2	76	14:31 (0:54)			
Sampling at 2–3 p.m.; day 2	102	14:58 (0:51)	33 (34)[2]	99	4.82 (2.68–9.15)
Child had meal; day 2	100	19:39 (1:11)			
Sampling before sleep; day 2	100	20:13 (2:03)		97	1.44 (0.90–3.44)
Child fell asleep; day 2	96	20:49 (2:08)			

[1] Number of subjects with information about their waking, sleeping and mealtimes.
[2] Calculated from the sub-population with reported afternoon naps. A total of 88.7% (n = 94) of the children in the out-of-home childcare group took naps on Monday, and 62.3% (n = 66) of them took naps on Sunday. Correspondingly, a total of 72% (n = 77) of the children in the at-home childcare took naps on Sunday, and 71% (n = 76) of the children took naps on Monday.
[3] Number of subjects with valid cortisol values, and missing values were caused by failed sampling.

Study II consisted of all the same children, who participated in Study I, except those who did not have their mother's report of Early Childhood Behavior Questionnaire ECBQ at the age of 2 years (n = 50). The total number of participants in Study II was 163 children of which 84 were attending out-of-home, center-based childcare, and 79 children were cared for at home. The mean age of the children was the same as in Study I by being 2.1 years old (SD 0.6). Similarly, the mean age of the children was higher in the out-of-home childcare group being 2.3 years old (SD 0.6), while in the at-home parental care group, the mean age of the children was 2.0 years old (SD 0.5). The proportion of boys and girls was in balance between the groups. In the out-of-home childcare group the number of boys was 50 and girls 34, while in the at-home parental care group, the number of boys was 40 and girls 39. The detailed daily schedule of the children and cortisol values are reported in the Table 3 from Original Publication II).

The primary caregivers in the at-home parental care group during the study participation were mothers (n = 69) and only a minority were fathers (n = 6), grandparents or relatives (n = 3) or other caregivers at home (n = 1). More than half (55.7%) of the children in the at-home parental care group had siblings at home concurrently, and the number of them varied from one (39.2%), two (11.4%) or three (5.1%) siblings at home during the study days.

The children were divided across 31 different childcare centers of which 15 were private, and 16 were public childcare units. Furthermore, the children were divided mostly across different groups within the childcare centers, and thus, not clustered in a particular center. Most children (n = 61) participated in full-time childcare and only a small number (n = 23) had part-time childcare. Full-time childcare included, on average, 20 childcare days within a month, and part-time childcare consisted of a maximum of 16 days (n = 20) or 11 days (n = 3) days within a month. The average group size in the childcare centers was 13.19 (SD 3.8) children, and the child-to-caregiver ratio was on average 4.47 (SD 1.1).

Table 3. Descriptive statistics of saliva sampling and cortisol (nmol/l) values in Study II.

		Sampling time	Time between wake up and sampling (minutes)	Raw cortisol values (nmol/l)	Log. -transform. cortisol values	Cortisol AUC_G[3]
	n[1]	*M (SD)*	*M (SD)*	*Median (Interquartile range)*	*M (SD)*	*M (SD)*[3]
Out-of-home childcare group (*n = 84*)						
Child woke up; day 1	78	07:37 (0:49)				5.91 (3.79)
Sampling after wake-up; day 1	75	08:04 (0:49)	27 (28)	10.91 (6.33–13.92)	1.0 (0.4)	
Sampling at 10 a.m.; day 1	80	10:09 (0:24)		3.17 (2.28–5.15)	0.6 (0.4)	
Sampling at 2–3 p.m.; day 1	78	14:46 (0:46)	38 (47)[2]	2.98 (1.96–4.60)	0.5 (0.4)	
Sampling before sleep; day 1	78	20:10 (0:52)		0.96 (0.65–2.39)	0.1 (0.5)	
Child fell asleep; day 1	79	20:52 (0:57)				
Child woke up; day 2	81	07:01 (0:31)				6.47 (2.54)
Sampling after wake-up; day 2	78	07:14 (0:34)	14 (19)	8.70 (5.90–13.30)	1.0 (0.3)	
Sampling at 10 a.m.; day 2	79	10:05 (0:14)		3.09 (2.35–4.11)	0.5 (0.2)	
Sampling at 2–3 p.m.; day 2	73	14:15 (0:28)	28 (30)[2]	3.95 (2.53–7.23)	0.6 (0.3)	
Sampling before sleep; day 2	79	20:10 (0:44)		1.10 (0.73–1.83)	0.1 (0.5)	
Child fell asleep; day 2	77	20:56 (0:50)				
At-home parental care group (*n = 79*)						
Child woke up; day 1	76	07:57 (1:08)				8.18 (5.55)
Sampling after wake-up; day 1	74	08:26 (1:00)	29 (34)	10.89 (7.06–19.78)	1.1 (0.5)	
Sampling at 10 a.m.; day 1	70	10:19 (0:37)		4.65 (3.14–7.52)	0.8 (0.5)	
Sampling at 2–3 p.m.; day 1	76	14:58 (0:54)	35 (36)[2]	4.10 (2.54–7.54)	0.7 (0.5)	
Sampling before sleep; day 1	71	20:19 (0:48)		1.55 (0.83–3.85)	0.4 (0.7)	
Child fell asleep; day 1	74	21:04 (0:48)				
Child woke up; day 2	77	07:42 (0:55)				8.39 (4.96)
Sampling after wake-up; day 2	72	08:01 (0:51)	19 (18)	10.17 (7.39–16.31)	1.1 (0.4)	
Sampling at 10 a.m.; day 2	78	10:19 (0:45)		4.65 (2.99–7.71)	0.8 (0.4)	
Sampling at 2–3 p.m.; day 2	74	14:52 (0:40)	31 (33)[2]	4.48 (2.56–10.52)	0.7 (0.5)	
Sampling before sleep; day 2	73	20:26 (0:46)		1.56 (0.93–4.78)	0.4 (0.7)	
Child fell asleep; day 2	70	20:59 (0:57)				

[1] Number of subjects with information of waking and sleeping times and valid cortisol concentration values; missing cortisol values were caused by failed sampling.

[2] Calculated from the sub-sample with reported afternoon naps. A total of 61% (n = 51) of the children in the out-of-home childcare group took naps on Sunday, and 87% (n = 73) of them took naps on Monday. Correspondingly, a total of 72% (n = 57) of the children in the at-home parental care took naps on Sunday, and 71%, (n = 56) of the children took naps on Monday.

[3] Calculated for all subjects (n = 84/79) using multiply imputed data.

When the same children who participated in Studies I and II approached the age of 3.5 years, the 217 originally recruited families were contacted again and asked to participate in the next measurement point of the study. Family situations usually change a lot at this age, but the families with a similar childcare arrangement than in the first measurements at the age of 2 years were eligible to participate. Altogether 111 originally recruited families participated in Study III. The mean age of the children was 3.6 years old (SD 0.1), and there were no differences in child ages between the groups (Table 5).

In the out-of-home childcare group, the 109 originally recruited families were contacted. The final sample of the children participating in out-of-home childcare consisted of 84 children, because 13 families were not interested in participating or for some other reason could no longer participate in the study. Three children were no longer attending out-of-home childcare, and 9 families were moved to another place and were not able to participate. The mean age of the children was 3.6 years (SD 0.1), and the proportion of boys was 48 and for girls, it was 36. A total of 32 childcare centers participated, half of which were public, and the other half were private centers. The average group size in the childcare centers was 18.29 (SD 3.8) children, and the child-to-caregiver ratio was on average 6.46 (SD 1.1). The participants were not clustered in particular centers, as most of the children participated in different childcare units or in different groups within the childcare center. Most children (n = 69) participated in full-time childcare, and only a small number (n = 15) had part-time childcare. Full-time childcare included on average 20 childcare days within a month, and part-time childcare consisted of a maximum 16 days (n = 13) or 11 days (n = 2) days within a month.

Most children, who were cared for at home during Studies I and II, had started to attend out-of-home childcare at the age of 3.5 years and were thus no longer eligible for the study. A total of 108 originally recruited families, who participated in the first phase of the study, were contacted again. On the whole, 27 children were still cared for at home and able to participate in Study III. A total of 70 originally recruited children had started to attend out-of-home childcare, 10 families were not interested in participating or could not participate for some other reason and one family had moved to another place. One child had attended for a short time in out-of-home childcare between measurement points but returned to at-home parental care and was thus eligible for the study. The mean age of the children was 3.6 years old (SD 0.1), and the number of boys was 13 and girls was 14. Most children were cared for at home by a parent (n = 25) and a small minority by a relative (n = 2). The number of siblings varied from one (77.8%), two (14.8%) or three (7.4%) siblings at home during the study participation.

Finally, there were three children with 1 to 2 extremely high cortisol values. For one child, the mother had reported medication and illness that possibly affected cortisol values. However, for others, the mother had not reported anything special concerning the sample taking. These samples were removed as outliers in our statistical analyses. We assumed that the samples were contaminated or had some other sources of unknown inaccuracy affecting the results, which could not be controlled for in this study. Furthermore, one child had a single sample, which was taken in a wrong place, and thus, did not follow the study protocol. That sample was also removed, and the rest of the samples were retained in our analyses. The detailed daily schedule and cortisol values are reported in Table 4 from Original Publication III.

Table 4. Descriptive Statistics of the diurnal cortisol values (nmol/l) in Study III.

	n^1	Sampling time *M (SD)*	Time between wake-up and sampling (minutes) *M (SD)*	Raw cortisol values (nmol/l) *Median (Interquartile range)*	Cortisol AUC_G *M (SD)*
Out-of-home childcare group (*n = 84*)					
Child woke up; day 1	77	07:42 (0:52)			5.02 (3.42)
Sampling after wake-up; day 1	75	08:11 (0:58)	30 (28)	8.04 (5.23–13.53)	
Sampling at 10 a.m.; day 1	79	10:17 (0:38)		3.03 (2.15–5.34)	
Sampling 2–3 p.m.; day 1	83	14:35 (0:34)	25 (17)[2]	2.53 (1.64–4.61)	
Sampling before sleep; day 1	82	20:16 (0:43)		0.82 (0.52–1.53)	
Child fell asleep; day 1	75	20:55 (0:44)			
Child woke up; day 2	83	06:59 (0:33)			5.64 (2.53)
Sampling after wake-up; day 2	82	07:13 (0:36)	15 (14)	8.77 (6.00–11.95)	
Sampling at 10 a.m.; day 2	79	10:09 (0:26)		2.89 (2.10–3.90)	
Sampling at 2–3 p.m.; day 2	80	14:11 (0:28)	29 (23)[2]	3.36 (2.12–4.94)	
Sampling before sleep; day 2	79	20:18 (0:38)		0.75 (0.52–1.74)	
Chid fell asleep; day 2	73	21:00 (0:36)			
At-home parental care group (*n = 27*)					
Child woke up; day 1	27	08:03 (0:52)			6.09 (5.13)
Sampling after wake-up; day 1	26	08:36 (0:48)	29 (30)	7.87 (5.16–18.86)	
Sampling at 10 a.m.; day 1	27	10:26 (0:49)		3.81 (2.74–7.84)	
Sampling at 2–3 p.m.; day 1	27	15:01 (0:40)	26 (18)[2]	2.75 (1.76–4.30)	
Sampling before sleep; day 1	25	20:38 (0:49)		0.87 (0.43–1.83)	
Child fell asleep; day 1	24	21:17 (0:40)			
Child woke up; day 2	27	07:43 (0:51)			6.69 (3.65)
Sampling after wake-up; day 2	27	08:05 (0:49)	22 (18)	10.17 (7.00–17.94)	
Sampling at 10 a.m.; day 2	27	10:56 (1:06)		3.49 (2.64–4.80)	
Sampling at 2–3 p.m.; day 2	26	15:15 (0:58)	41 (51)[2]	2.92 (2.00–4.45)	
Sampling before sleep; day 2	25	20:27 (0:50)		1.48 (0.56–5.19)	
Child fell asleep; day 2	21	21:07 (0:48)			

[1] Number of subjects with information of waking and sleeping times and valid cortisol concentration values; missing cortisol values were caused by failed sampling.
[2] Calculated from the sub-sample with reported afternoon naps. A total of 21.4% (n = 18) of the children in the out-of-home childcare group took naps on Sunday, and 84.5% (n = 71) of them took naps on Monday. Correspondingly, a total of 29.6% (n = 8) of the children in the at-home parental care reported naps on Sunday, and 18.5% (n = 5) of the children reported naps on Monday.

4.1.1 Background information

The background data of the mothers (i.e., age, education and origin) were determined from the FinnBrain Birth Cohort questionnaires during the pregnancy and the Medical Birth Register of the Finnish National Institute for Health and Welfare. Parents filled in a form describing their child's childcare history and daily rhythm, such as the child's waking time in the morning and their time of afternoon naps, their sleeping time during the evening, and their mealtimes as well as illnesses and prescribed medication on the saliva collection days. Childcare personnel filled out corresponding information about sleeping and mealtimes during the childcare day.

Childcare personnel also reported the group sizes and the number of caregivers in the childcare centers.

All the participants were ethnically Caucasian, and the mother's origin in 98.5% and language 97.1% of the cases were primarily Finnish. The mother's age at childbirth was on average 31.5 (SD 4.3) years old in both childcare groups. Maternal education was rather high, as 48.4% of the mothers had university-level education. Furthermore, maternal education was higher in the out-of-home childcare group at 2 years measurement, while no significant difference between the groups was observed at the age of 3.5 years. The proportion of boys and girls was in balance between the childcare groups. The mean age of the children was higher in the first phase of the study in the out-of-home childcare, while no age difference between the groups appeared in the second phase of the study. Originally, a total of 32 childcare centers participated in the study of which 18 were public and 14 were private childcare units. The length of the out-of-home childcare attendance at the age of 2 varied between less than 2 weeks and two years with a mean of 9.7 months (SD 7.3). At the age of 3.5, the mean length of childcare attendance was 25.9 months (SD 6.6). Most children had full-time care, which included on average 20 childcare days within a month, and only a minority had part-time care. The average groups size in the childcare centers for children at 2 years was 13.47 (SD 3.8), and the child-to-caregiver ratio was 4.59 (SD 1.2). At the age of 3.5 years old, the average group size in the childcare centers was 18.29 (SD 3.8) children, and the child-to-caregiver ratio was on average 6.46 (SD 1.1).

The main caregiver during the measurement days in the at-home parental care group was the child's own parent. For more than 90%, the caregiver at home was a mother or a father and only for a minority a grandparent or other caregiver at home. Most children also had siblings, and the number of siblings varied from one (41.1%), two (11.2%) or three (4.7%) siblings at the age of 2, and one (77.8%), two (14.8%) or three (7.4%) siblings at the age of 3.5 at home during the study days.

Finally, at either age point, no group differences in the levels of temperament traits were observed. Demographic characteristics of the participants are presented in Table 5.

Table 5. Demographic characteristics of the participants at 2 and 3.5 years of age.

	Out-of-home childcare	At-home parental care	Total sample	*P-value*
2 years				
Sample *n*	106	107	213	
Child age (years), mean (SD)	2.26 (0.6)	2.00 (0.5)	2.13 (0.6)	.001
Child sex (boys), *n* (%)	63 (59.4%)	53 (49.5%)	116 (54.5%)	.147
Child Temperament, mean (SD)[1]				
Surgency/Extroversion	5.1 (0.6)	5.1 (0.6)	5.1 (0.6)	.962
Negative affectivity	2.9 (0.6)	3.0 (0.6)	2.9 (0.6)	.280
Effortful control	5.0 (0.6)	5.0 (0.5)	5.0 (0.6)	.660
Maternal characteristics				
Maternal education *n* (%)				
High school / Vocational education	16 (15.1%)	29 (27.1%)	45 (21.1%)	.048
Applied university	31 (29.2%)	34 (31.8%)	65 (30.5%)	
University degree	59 (55.7%)	44 (41.1%)	103 (48.4%)	
Maternal income n (%)				
Low < 1500 eur	36 (33.9%)	42 (39.3%)	78 (36.6%)	.145
Med 1501-2500 eur	55 (51.9%)	58 (54.2%)	113 (53.1%)	
High > 2501 eur	15 (14.2%)	7 (6.5%)	22 (10.3%)	
3.5 years				
Sample *n*	84	27	111	
Child age (years), mean (SD)	3.60 (0.1)	3.56 (0.1)	3.59 (0.1)	.057
Child sex (boys), *n* (%)	48 (57.1%)	13 (48.1%)	61 (55%)	.414
Child Temperament, mean (SD)[2]				
Surgency/Extraversion	5.1 (0.6)	5.0 (0.7)	5.1 (0.6)	.521
Negative affectivity	2.8 (0.6)	3.1 (0.5)	2.9 (0.6)	.097
Effortful control	5.1 (0.6)	5.0 (0.5)	5.0 (0.5)	.550
Maternal characteristics				
Maternal education *n* (%)				
High school / Vocational education	12 (14.3%)	9 (33.3%)	21 (18.9%)	.073
Applied university	22 (26.2%)	7 (25.9%)	29 (26.1%)	
University degree	50 (59.5%)	11 (40.7%)	61 (55.0%)	
Maternal income *n* (%)				
Low < 1500 eur	23 (27.4%)	13 (48.1%)	36 (32.4%)	.222
Med 1501-2500 eur	48 (57.1%)	13 (48.1%)	61 (55.0%)	
High > 2501 eur	13 (15.5%)	1 (3.7%)	14 (12.6%)	

P values based on t-test for age, child temperament and χ^2 test for gender, income and education.
[1] Measured at the age of 2 and is based on n = 84 for out-of-home childcare and n = 79 for at-home parental care.
[2] Measured at the age of 2 and is based on n = 68 for out-of-home childcare and n = 21 for at-home parental care.

4.2 Child temperament assessment

Child temperament was evaluated at the age of two using maternal reports of Rothbart's Early Childhood Behavior Questionnaire (ECBQ) (Putnam et al., 2006). The ECBQ is widely used and has 107 questions. It is a valid and reliable questionnaire for assessing temperament in children between 18 to 36 months of age. Mothers answered questions using a seven-point Likert-style scale about how often they had observed a particular child behavior during the past two weeks. The questionnaire contains three main factors of temperament: negative affectivity, surgency or extroversion and effortful control. Internal consistency scores of the factors in Study II were as follows: negative affectivity (Cronbach's α = .914), surgency or extroversion (α = .832) and effortful control (α = .876) and in Study III: negative affectivity (α = .889), surgency or extroversion (α = .839) and effortful control (α = .858). For all scales, higher scores reflected higher level of the particular temperament characteristic in question.

4.3 Saliva cortisol collection

Cortisol can be measured, for instance, from blood, urine, saliva and hair (Koss & Gunnar, 2018). The assessment of cortisol in saliva is a widely used method in psychoneuroendocrinology research. Due to its non-invasiveness, it is a very useful method particularly for young children (Kirschbaum & Hellhammer, 1994). As the day-to-day cortisol production follows a circadian rhythm, endocrinological research often includes repeated cortisol measures to examine the changes in cortisol excretion during a day (Gunnar & Quevedo, 2007). Studies assessing diurnal variation normally include a collection of multiple samples at waking, the CAR and evening hours to examine different aspects of the HPA axis functioning. Measurements over multiple days allow studying day-to-day variation, while a single measure of cortisol is almost meaningless (Koss & Gunnar, 2018).

Children's saliva samples were collected over two days, four samples during each day being 30 minutes after waking up in the morning, at 10 a.m., between 2–3 p.m. and in the evening before going to sleep. The sample collection was designed to cover the whole day from awakening until bedtime in both childcare groups and in both measurement days. The first day of collection was Sunday, when the children in both childcare groups were at home. The second day of collection was Monday, when the other group of the children spent their day in at-home parental care, and the other group was attending out-of-home, center-based childcare. Two samples were collected during the out-of-home childcare day in order to examine the difference between the mid-morning (10 a.m.) and mid-afternoon (2–3 p.m.) cortisol values. Most parents picked-up their children from childcare between 4–5 p.m., and the afternoon sample collection was implemented before that. Some children had part-time care, which meant a limited number of days per month in childcare. However, children in part-time care attended full days during their childcare days enabling both mid-morning and mid-afternoon saliva sampling in the out-of-home childcare setting. For eight children (Study I), six children (Study II) and five children (Study III), the samples were not taken on Monday, because the children did not attend out-of-home childcare on Mondays. However, the samples were collected immediately during the next childcare day after the day-off.

The parents collected saliva samples at home, and the childcare personnel collected samples in the childcare center. The person, who collected the samples in the out-of-home childcare center, was the child's own caregiver, and thus, familiar with the child. The high quality of samples was ensured by the research nurse who taught the parents and the childcare personnel to properly take the samples. In addition, parents and childcare personnel were given written information and a tutorial video. Furthermore, parents and childcare personnel were advised that the children should not do physical activity for thirty minutes and eat for fifteen minutes before sampling. The saliva samples were collected using Salimetrics© infant swabs (Stratech, Suffolk, UK) by keeping the polymer swab in the child's mouth for two minutes during the collection. The participating families were given small gifts for their children such as stickers or toys in thanks for the effort.

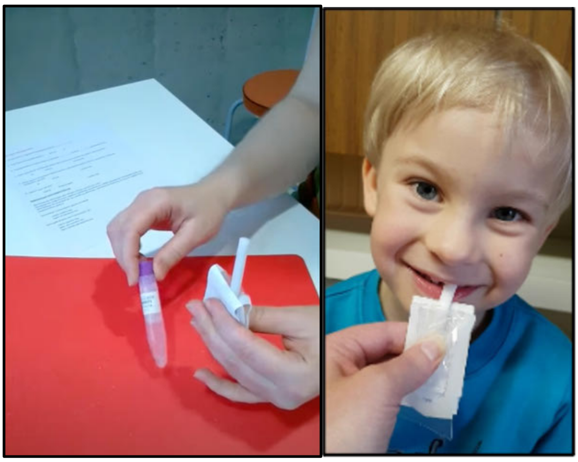

Figure 4. Saliva samples were collected using a polymer swab.

4.3.1 Sample storage

Saliva samples were placed in the swab storage tubes and kept in a refrigerator from two to five days between sample taking and delivery to the research center. An inter-laboratory stability test for cortisol in saliva verified that samples remained stable at room temperature for at least seven days, and the storage did not have an effect on the measurement (Jensen et al., 2014). For most of the study period, samples were collected immediately from homes and childcare centers by a research assistant, but a small number of the samples, at the beginning of the study, were returned by mail within five days of collection. After delivery, the saliva samples were immediately centrifuged (4°C, 15 min, 1800 x g) and frozen at -70°C. The samples were analyzed by The Finnish Institute of Occupational Health research laboratory, which regularly participates in the international quality control. The free cortisol in saliva was analyzed using the Cortisol Saliva Luminescence Immunoassay (RE62111, IBL-International, Germany). The linear reportable range of the assay was 0.276–86.4 nmol/l. The coefficient of the variation for the intra- and inter-assay of the method was 5% and 8%, respectively.

4.4 Statistical analyses

The diurnal cortisol values can be modeled for instance by calculating the area under the curve (AUC) or by modeling the diurnal cortisol slopes, which characterize slightly different aspects of the HPA axis activity (Pruessner et al., 2003; Saridjan et al., 2014). The AUC is a widely used measure when examining the associations between repeated measures in multiple time points and other variables. In endocrinological studies, the AUC is often used to estimate circadian changes of cortisol hormones or to assess the overall cortisol secretion over a specific time period. The area under the curve, with respect to ground AUC_G, is more related to total hormonal output, while the area under the curve with respect to an increase AUC_I is related to changes over time (Pruessner et al., 2003). Thus, the AUC represents the overall activity of the HPA axis throughout the day but discards information about the diurnal variation. Modeling the diurnal cortisol slope, in turn, is an appropriate measure, when the interest is in the cortisol decline over the day or in the values of a specific time frame within a day (Adam & Kumari, 2009; Rotenberg et al., 2012). In this study, we have examined both the diurnal cortisol slopes by analyzing the shapes of the diurnal cortisol profiles (Studies I and III) and also the total diurnal cortisol production AUC_G in order to analyze the overall activity of the HPA axis during a day (Studies II and III).

The log-transformed (base 10) cortisol values were used in the analyzes, because the distribution of the original values was positively skewed. In all the studies, a significance level of p-values < .05 was considered as a significant result, and 95% confidence intervals were reported. All the statistical analyses were performed in R (R Core Team, 2018) with the following packages: nlme (Pinheiro et al., 2018) for fitting the mixed models, robustmll (Koller, 2016) for robust analysis and ggplot2 (Wickham, 2009) for the figures.

4.4.1 Study I

The main goal in Study I was to compare diurnal cortisol profiles between children participating in out-of-home childcare and children who were cared for at home. First, “the time since wake up” for each child was calculated separately in order to make the diurnal cortisol slopes/profiles comparable with each other. The families were instructed to take saliva samples approximately 30 minutes after a child waked up in the morning, because the cortisol values normally peak (Cortisol awakening response, CAR) approximately 30–45 minutes after the wake up (Bäumler et al., 2013). However, the sampling times varied a little between the children, as it normally does in the home sampling procedures, and the cortisol peak could not be reached in all the children. Therefore, the time across the entire day starting from the individual waking up time in the morning was modeled for each child and used as the starting point ($t = 0$) for each child's diurnal sample collection. Continuous time since awakening, instead of a general time of the day, was used as the time variable in the analyses. "The time since wake up" was calculated by subtracting the wake-up time from the cortisol measurement time. There were also some missing time values at each cortisol measurement point, and the missing time values were imputed by the median measurement time, since wake up, at that particular cortisol measurement point.

Also, the effect of the naps for mid-afternoon cortisol values was controlled for by a three-class variable indicating how long after waking up from the naps the saliva samples were taken. The possible values were < 15 minutes, between 15 and 60 minutes or over 60 minutes after wake up/no naps at all. The mid-afternoon sampling was not anchored with the naps, because the timing of the naps varied between children and not all the children took naps. A total of 62.3% of the children in the out-of-home childcare group and 72% of the children in at-home parental care were reported to take naps on Sunday. Correspondingly, a total of 88.7% of the children in the out-of-home childcare group and 71% of the children in the at-home parental care reported taking naps on Monday.

The children's saliva cortisol levels were then modeled using a linear mixed effects model. The main aim was to analyze whether the diurnal cortisol levels differed between the childcare groups, i.e., out-of-home childcare or at-home parental care, and also between the measurement days, i.e., Sunday or Monday. Therefore, the binary variables, "group" and "day," were included in the model. The diurnal cortisol profile, i.e., the relation between the cortisol value and time, since wake up, was modeled by a natural cubic spline (de Boor, 1978) with cut off points at 2 hours and 44 minutes and 7 hours and 10 minutes, i.e., at the median time values at the second (10 a.m.) and third (2–3 p.m.) cortisol measurement points. Furthermore, the maternal education, maternal income level, child's age and sex were controlled for in the model.

The child-specific effects, i.e., random effects, used in the model were chosen by testing a few sensible options and then choosing the model that had the lowest Akaike information criterion (AIC) (Akaike, 1987). By this means, a result was achieved that included in the model a linear time effect, i.e., random slope, and separate random intercepts for Sunday and Monday for each child.

The model was then fitted with only the main effects to find out how the cortisol levels differed between the childcare groups, i.e., out-of-home childcare or at-home parental care. After this, the difference in mid-afternoon cortisol levels, defined as 7 hours and 10 minutes since waking up in the morning, between the measurement days, i.e., Sunday and Monday, in both childcare groups were examined. This was done by fitting a model including the interactions among the group, the day and time spline terms and the above-mentioned main effects and then using the model to estimate the mid-afternoon cortisol differences. The standard errors for these differences were estimated by bootstrapping the model using 1000 bootstrap samples.

Finally, the main effects model for only the out-of-home childcare group was fitted. The model included variables for the duration of childcare attendance; the group size in a childcare center and the childcare form, part-time or full-time care, to examine whether these variables were associated with cortisol levels in the out-of-home childcare setting.

As some of the children had exceptionally high cortisol levels, the main effects models, using robust regression analysis, were also fitted. The robust regression gives less weight to the extreme values, and therefore, the results were less sensitive to extreme observations. The random intercept was the only random effect in the robust regression models.

4.4.2 Study II

In Study II, the area under the curve with respect to ground AUC_G was used to model the total diurnal cortisol production of the children. The AUC_G was chosen, because there was not a specific interest to explore variation in the circadian rhythm or cortisol values in a specific time window. Instead, the aim was to investigate the overall intensity of HPA axis functioning and total cortisol output during the measurement days and the associations with temperament characteristics. The AUC is widely used in studies combining child individual characteristics and cortisol production. For instance, the associations between a toddler's cognitive functioning (Saridjan et al., 2013) and diurnal cortisol output as well as sleep patterns in preschoolers (Saridjan et al., 2017) have been examined through computation of AUC. The AUC calculation was also used in the study of Suhonen et al. (2016) examining the associations between temperament, cognitive functions, language skills and total diurnal cortisol production in 2-year-old children attending out-of-home childcare.

Figure 5, from Original Publication II, illustrates in a detailed way, how the cortisol values of the first measurements in the morning were defined, and how the AUC_G was calculated in this study. First, morning saliva cortisol measurements were made comparable between participating children, and the “time since wake up” instead of absolute time, was used as the time variable. The participating families were instructed to take first saliva samples in the morning approximately 30 minutes after the child’s wake up, because the cortisol values are highly dependent on the time of waking. Cortisol values typically have a peak approximately 30–45 minutes after the wake up in the morning (Bäumler et al., 2013). However, the sampling times in participants varied between 0 and 180 minutes after waking up, and therefore, the predicted 30-minute cortisol value was estimated for every child. These estimations were made by first fitting a LOESS regression curve to approximate the mean cortisol curve during the first few hours and then using this curve to predict the cortisol values at 30 minutes after waking up. Thereafter, these estimations were used through the analyses.

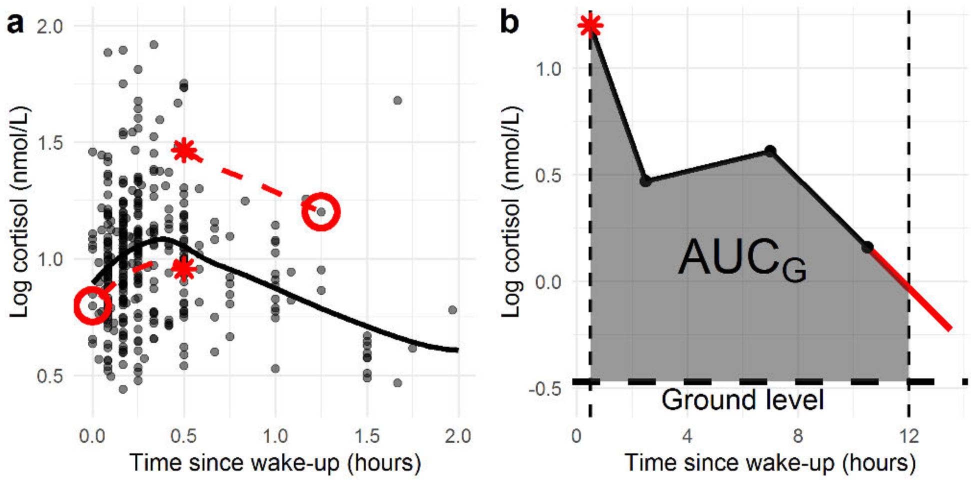

Figure 5. Illustrations of how the 30-min cortisol values were estimated, and how the AUC_G was defined in Studies II and III.

a) Illustration of how the predicted 30-min cortisol values were estimated to achieve a comparable starting point to every individual. The solid black line is the estimated LOESS curve representing the average cortisol curve during the first hours after wake up. The 30-min cortisol estimation is shown for two examples: the original observations are within the red circles, and the corresponding predicted 30-min cortisol values are marked by a red star.

b) Definition of AUC_G. The red line represents the estimated cortisol curve for a child, whose last saliva sample was taken before the 12-hour time period had been reached.

There were also some missing cortisol values in our data (Table 2), and the predictive mean matching multiple imputation technique (Little, 1988) was used to construct 100 complete data sets. The imputation model included the measurement time, cortisol values and all the predictors in the analysis models and variables indicating afternoon naps. After the imputation, AUC_G for the time interval from 30 minutes to 12 hours after waking in the morning was calculated for each subject for each day and separately in each completed dataset. For those children whose last measurement was before the 12-hour time limit, the last line in the cortisol curve approximation was linearly continued to the 12-hour limit (the red line in Figure 5, b). The definition for AUC_G was similar to the definitions given by Fekedulegn et al. (2007) with the exceptions that a) the lower and upper bound of the time interval was set to a fixed time, i.e., waking and 12 h after the waking that did not necessarily correspond to any measurement time points and b) as the log-transformed cortisol values was used, the ground level was not any true zero level but an arbitrarily chosen fixed level. The choice of the ground level, however, did not affect the results of the analyses apart from the intercept term in the regression models.

The reliability of the AUC_G measures was estimated by calculating the Pearson correlations between the AUC_G values on Sunday and Monday. The correlations were calculated for both the whole sample and separately for the two distinct childcare groups.

A multilevel model with a random intercept per child and the following fixed effect structure was used to analyze the associations between cortisol AUC_G and the child temperament variables in the whole population.

Model 1: AUC_G = Surgency/Extroversion + Negative affectivity + Effortful control + Group + Measurement day + Sex + Age + Maternal Education.

The variables controlled for in the model were: group (out-of-home childcare or at-home parental care), measurement day (Sunday or Monday), child sex (boy or girl), age (years) and maternal education (low, mid or high).

To investigate how the temperament traits moderate the AUC_G differences between the groups, the weighted least squares regression (WLS) models with the following structure were used.

Model 2: $AUC_{G(Mon/Sun)}$ = Surgency/Extroversion + Negative affectivity + Effortful control + Group + (Surgency/Extroversion + Negative affectivity + Effortful control) × Group + Sex + Age + Maternal Education.

Separate models were used for Sunday, a home day for both childcare groups, and Monday, an out-of-home day for the out-of-home childcare group. $AUC_{G(Mon/Sun)}$ in the formula means either AUC_G on Sunday or AUC_G on Monday. To take into account the clearly different variances in AUC_G between the at-home parental care and the out-of-home childcare groups (Table 3), the variances of the random effects (Model 1) or the variances of the residuals (Model 2) were *not* assumed equal between the groups.

The separate models for Sunday and Monday were chosen, because the power of the study was too low to reliably conduct a more complex "group x temperament x day" interaction. In addition, the primary goal focused on the question about whether child temperament would moderate the association between childcare contexts and total diurnal cortisol production, but not the moderating effect of the day or differences per measurement day as such. However, the day is an inherent part of the design, because the environment is expected to be more clearly different between the childcare groups on Monday than on Sunday, and thus, separate analyses of the measurement days may reveal associations that could not be observed by collapsing the data from these two weekdays together. Therefore, the days were analyzed with separate models when investigating the interaction between child temperament and childcare context, even though this design does not allow for conclusions related to actual differences per day.

All the analyses were first performed on each imputed dataset, and the final results were then obtained by pooling all the results using Rubin's rules (D. B. Rubin, 1987). All statistical analyses were performed in R 3.5.2 (R Core Team, 2018) with the packages mice (van Buuren & Groothuis-Oudshoorn, 2011) for multiple imputation, nlme (Pinheiro et al., 2018) for WLS regression and multilevel modeling and ggplot2 (Wickham, 2009) for the Figures 5 and 8.

4.4.3 Study III

The statistical analyzes of Study III were equal to the analyzes made in Studies I and II. The main aim in Study III was to examine whether associations between the childcare groups and temperament and measurement days retain or change as the children develop from 2 to 3.5 years of age. The area under the curve with respect to ground (AUC_G) was used as the measure of total diurnal saliva cortisol (Pruessner et al., 2003). The formulation of AUC_G for this Study III was equal to the method used in Study II and is presented in Figure 5 from Original Publication II.

The AUC_G values for each child in each study question regarding the total cortisol production were analyzed using a multilevel model with a random intercept for each child. The fixed effects of the models varied by study question and were as follows:

Total diurnal cortisol production between children in out-of-home childcare and children in at-home parental care groups:

Model 1: $AUC_G = Group + Day + Sex + Age + Education$

Total diurnal cortisol production between the measurement days (Sunday and Monday) separately in both groups:

Model 2: $AUC_G = Day + Sex + Age + Education$

Associations between each temperament trait and total diurnal cortisol production:

Model 3: $AUC_G = Temperament + Group + Day + Sex + Age + Education$

The predictor variables in the models were: *Group* (out-of-home childcare or at-home parental care), measurement *Day* (Sunday or Monday), child *Sex* (boy or girl), *Age* (years) and *Temperament* (Surgency/Extroversion, Negative affectivity or Effortful control) and maternal *Education* (High school / Vocational education, Applied university or University degree). The variances of the random intercepts were *not* assumed equal between the childcare groups.

Post-hoc analyses were performed to examine the age dependency related to the difference in AUC_G values between the childcare groups and the association between Surgency/Extroversion and the AUC_G values. The analyzes were based on the data from the 2-year measurement point (Study I) and the current 3.5-year measurement point (Study III). Multilevel models with two random intercepts (one per each age point) and the following fixed effect structures were used to analyze these age interactions:

Model 1: $AUC_G = Group + Age + Group \times Age + Day + Sex + Education$

Model 3: $AUC_G = Surgency + Age + Surgency \times Age + Group + Day + Sex + Education$

Finally, the differences in afternoon cortisol levels between Sunday and Monday in both childcare groups at the age of 3.5 years were analyzed. The method used in the analyzes was practically the same as in Study I at 2 years of age. That is, children's saliva cortisol levels were modeled using a multilevel model with two random intercepts, one for each day and a random (time) slope for each child and the following fixed effects structure:

$$Log(cortisol) = Group + Day + TimeTerms + Group \times Day + Group \times TimeTerms + Day \times TimeTerms + Group \times Day \times TimeTerms + Napping + Age + Sex + Education$$

Similar to Study I, the *TimeTerms* refer to the terms of the natural cubic spline that was used to model the dependency of cortisol levels on the time since awakening. Furthermore, the effect of afternoon naps on the afternoon measurements was controlled for by using a three-class variable indicating how long after the naps the cortisol samples were taken.

All statistical analyses were performed in R 3.6.3 (R Core Team, 2018) with the packages mice (van Buuren & Groothuis-Oudshoorn, 2011) for multiple imputation and nlme (Pinheiro et al., 2018) for fitting the multilevel models.

4.5 Ethical consideration

This research meets the ethical guidelines and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The Ethics Committee of the Hospital District of Southwest Finland approved "The FinnBrain Birth Cohort Study" on 14.6.2011 with the protocol number: ETMK: 57/180/2011. This research entitled: "The Quality of Day Care and the Risk of the Social Exclusion in Early Childhood" was approved by The Ethics Committee of the Hospital District of Southwest Finland on 26.11.2013 with the protocol number: ETMK: 137/1801/2013. All the study participants gave their written informed consent, and parents gave consent on behalf of their child. The participation was voluntary, and the study subjects had the right to interrupt participation at any time without giving any specific reason.

5 Results

5.1 Diurnal cortisol profiles in different childcare settings (Study I)

The results in Study I indicated that the total diurnal cortisol profiles at the age of 2 were similar in both childcare groups and in both measurement days, which followed a typical circadian rhythm, where the cortisol levels are highest after waking up in the morning and decline towards the evening (Figure 6). However, the overall saliva cortisol levels (nmol/l) were 30% higher (95% CI: [9%, 54%], p = .004) in the at-home parental care group when compared to out-of-home childcare group. Maternal education, income level, child sex and age were controlled for in the models, and they did not explain the differences between the groups.

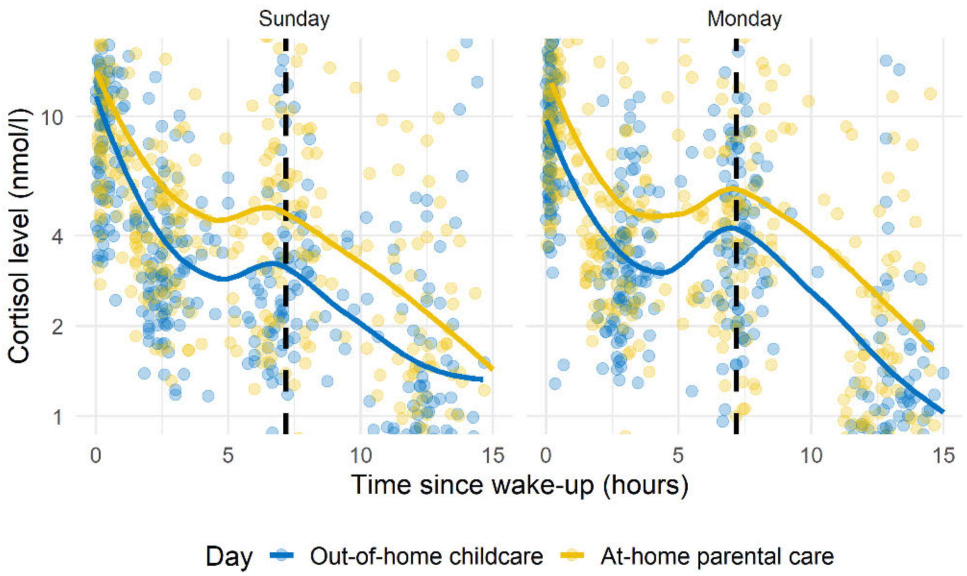

Figure 6. Diurnal cortisol profiles in both childcare groups and in both measurement days (Sunday and Monday).

A slight increase in the diurnal cortisol levels was observed in both childcare groups and in both measurement days during the mid-afternoon. The increased mid-afternoon cortisol levels were partly explained by the afternoon naps. About 35% of the mid-afternoon samples were taken 15–60 minutes after the naps, which was found to be associated with 46% higher cortisol levels ([24%, 71%], $p < .0001$) when compared to cortisol levels measured over 60 minutes after waking up or in children who had not napped at all (Table 6a, from Original Publication I).

Table 6a. The parameter estimates and the corresponding standard errors and p-values for the fixed effects from the main effects models in Study I. The reference classes of the categorical variables are in parenthesis. The response variable is a base 10 logarithm of the cortisol level. Therefore, the proportional effect of each predictor on the cortisol level (expressed in the original units, nmol/l) is calculated as 10^B (B = the unstandardized regression coefficient).

Standard analysis Variable	Parameter estimate (B)	Standard error	*P*	Relative change (10^B)
(Intercept)	1.30	0.10	< .0001	19.74
Group (ref = out-of-home childcare)	0.11	0.04	.0047	1.30
Day (ref = Sunday)	0.01	0.02	.40	1.03
Time spline terms				
Term 1	-0.13	0.05	.014	0.73
Term 2	-1.35	0.05	<.0001	0.04
Term 3	-0.73	0.04	<.0001	0.18
Naps (ref = no naps / >60 min				
< 15 min	-0.03	0.04	.41	0.93
15–60 min	0.16	0.03	<.0001	1.46
Age	-0.08	0.04	.029	0.84
Sex (ref = girl)	0.01	0.04	.89	1.01
Education (ref = low)				
Mid	-0.13	0.06	.016	0.74
High	-0.06	0.05	.24	0.87
Income (ref = low)				
Mid	-0.07	0.04	.12	0.86
High	-0.01	0.07	.85	0.97
Duration of childcare attendance (years) [1]	-0.05	0.04	.25	0.89
Childcare form (ref = part-time care)[1]	0.05	0.04	.27	1.12
Group size in childcare[2]	-0.003	0.006	.64	0.99

[1] From the model fitted only to the data on the out-of-home childcare group.

[2] From a model fitted to the data on only 96 children (due to some missing data) in the out-of-home childcare group.

5.1.1 Afternoon cortisol levels in out-of-home childcare

When examining the childcare groups separately in Study I, the results indicated that the afternoon cortisol levels were 27% ([2%, 57%], p = .031) higher in the out-of-home childcare group during their childcare day when compared to the day children spent at home. The duration of childcare attendance (p = .25); the group size in the childcare center (p = .64) or the childcare form, part-time childcare or full-time childcare (p = .27) were not associated with cortisol levels. Finally, a robust analysis suggested that the children attending the full-time childcare, 20 days per month, had 23% higher cortisol levels ([4%; 44%]; p = .015) than the children attending the part-time care, with a maximum of 16 days per month) (Table 6b, from Original Publication I). In the at-home parental care group, differences in mid-afternoon cortisol levels between the measurement days were not found (20% [-4%, 48%]; p = .077) (Figure 7).

Table 6b. The Robust analysis.

Robust analysis Variable	Parameter estimate	Standard error	*p*[1]	Relative change (10^B)
(Intercept)	1.08	0.09	< .0001	11.91
Group (ref = out-of-home childcare)	0.09	0.03	.0061	1.23
Day (ref = Sunday)	0.03	0.01	.059	1.06
Time spline terms				
Term 1	-0.15	0.05	.0023	0.72
Term 2	-1.4	0.04	< .0001	0.04
Term 3	-0.81	0.03	< .0001	0.15
Naps (ref = no naps / >60 min				
< 15 min	-0.02	0.04	.56	0.95
15 – 60 min	0.17	0.03	< .0001	1.48
Age	-0.04	0.03	.18	0.91
Sex (ref = girl)	0.02	0.03	.60	1.04
Education (ref = low)				
Mid	-0.01	0.05	.74	0.97
High	0.01	0.04	.79	1.03
Income (ref = low)				
Mid	-0.04	0.03	.28	0.92
High	-0.03	0.06	.56	0.93
Duration of childcare Attendance (years) [2]	-0.003	0.04	.93	0.99
Childcare form (ref = part-time care)[2]	0.09	0.04	.015	1.23
Group size in childcare[3]	-0.002	0.005	.69	1.00

[1] The parameter estimators are assumed to be normally distributed.
[2] From the model fitted only to the data on the out-of-home childcare group.
[3] From a model fitted to the data on only 96 children (due to some missing data) in the out-of-home childcare group.

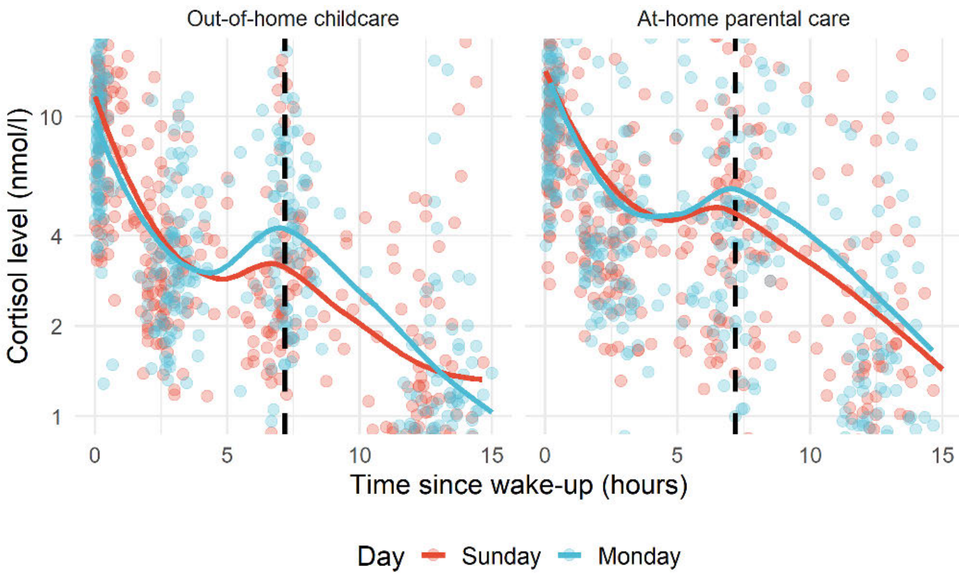

Figure 7. Differences in afternoon cortisol values between the measurement days (Sunday and Monday).

5.2 Moderating role of temperament in the association between a childcare setting and diurnal cortisol production (Study II)

The saliva samples were collected across two days per child at eight measurement time points. Cortisol levels may vary from day to day as well as the normal variation in this age group is large (Watamura et al., 2004). Hence, we first calculated the correlations with AUC_G values between the measurement days.

The results in Study II indicated a sound correlation between the days, i.e., Sunday and Monday in cortisol AUC_G values. Furthermore, the correlation was clearly higher in the at-home parental care group compared to the out-of-home childcare group, which most likely reflects the fact that Sunday and Monday are more similar among the children in at-home parental care compared to the children in out-of-home childcare.

The correlations [95% CI] were:
The whole sample: r = .74 [.66; .81] (Pearson); r = .65 [.54; .73] (Spearman)
Out-of-home childcare group: r = .50 [.30; .66]; r = .55 [.36; .69]
At-home parental care group: r = .82 [.73; .89]; r =.76 [.64; .84]

The results from the multilevel model in Study II are presented in Table 7 from the Original Publication II. The total diurnal cortisol production AUC_G at the age of 2 was higher in the at-home parental care group when compared to the children participating out-of-home childcare ($p = .002$). This is in line with our earlier results in Study I, where the overall cortisol levels were higher in children, who were cared for at home. In the whole study population, a higher level of the temperament trait surgency/extroversion was associated with higher total diurnal cortisol AUC_G ($p = .003$). However, no evidence was found for the associations between effortful control ($p = .13$) or negative affectivity ($p = .58$) and diurnal cortisol AUC_G. Furthermore, child sex, age or maternal education was not related to cortisol AUC_G.

Table 7. The results from the multilevel model (Model 1) in Study II. Child total diurnal saliva cortisol (AUC_G) modeled across the days (Sunday and Monday) and childcare groups (out-of-home childcare and at-home parental care).

Variable	Parameter estimate	Standard error	P-value
Intercept	4.53	3.94	.25
Neg. aff.	-0.27	0.49	.58
Eff. control	-0.76	0.50	.13
Surgency	1.30	0.44	.003
Group[1]	2.08	0.67	.002
Day[2]	0.39	0.27	.15
Age	-0.24	0.50	.63
Sex[3]	0.25	0.56	.65
Education (Mid)[4]	-0.44	0.91	.63
Education (High)[4]	-0.07	0.83	.94

[1] The reference level is “Out-of-home childcare.” [2] The reference level is “Sunday.” [3] The reference level is “Girls.” [4] The reference level is “Low.”

The results from Model 2 showed that the interaction terms between the temperament variables and childcare group were not significant either on Sunday or Monday in the stratified analyses (Table 8 and Figure 8 from Original Publication II). That is, we found no evidence for temperament as a moderator in the association between childcare setting and cortisol AUC_G.

Table 8. The results of the WLS regression analyses (Model 2) in Study II. Child total diurnal saliva cortisol (AUC_G) was modeled separately on Sunday and Monday.

Sunday				**Monday**			
Variable	**Parameter estimate**	**Standard error**	**P-value**	**Variable**	**Parameter estimate**	**Standard error**	**P-value**
Intercept	2.68	5.94	.65	Intercept	9.44	4.06	.020
Neg. aff.	0.14	0.79	.86	Neg. aff.	-0.34	0.54	.52
Eff. control	-0.71	0.77	.36	Eff. control	-1.16	0.53	.028
Surgency	1.32	0.68	.052	Surgency	0.89	0.47	.057
Group[1]	-8.98	11.08	.42	Group[1]	-4.44	9.52	.64
Age	-0.28	0.66	.67	Age	-0.31	0.48	.52
Sex[2]	0.38	0.73	.60	Sex[2]	0.22	0.54	.69
Education (Mid)[3]	0.04	1.17	.98	Education (Mid)[3]	-0.55	0.88	.54
Education (High)[3]	0.17	1.06	.87	Education (High)[3]	-0.04	0.8	.97
Neg. aff × Group	-0.71	1.37	.61	Neg. aff × Group	-0.39	1.15	.73
Eff. control × Group	1.33	1.42	.35	Eff. control × Group	0.76	1.21	.53
Surgency × Group	1.31	1.26	.30	Surgency × Group	0.73	1.08	.50

[1] The reference level is “Out-of-home childcare.”
[2] The reference level is “Girls.”
[3] The reference level is “Low.”

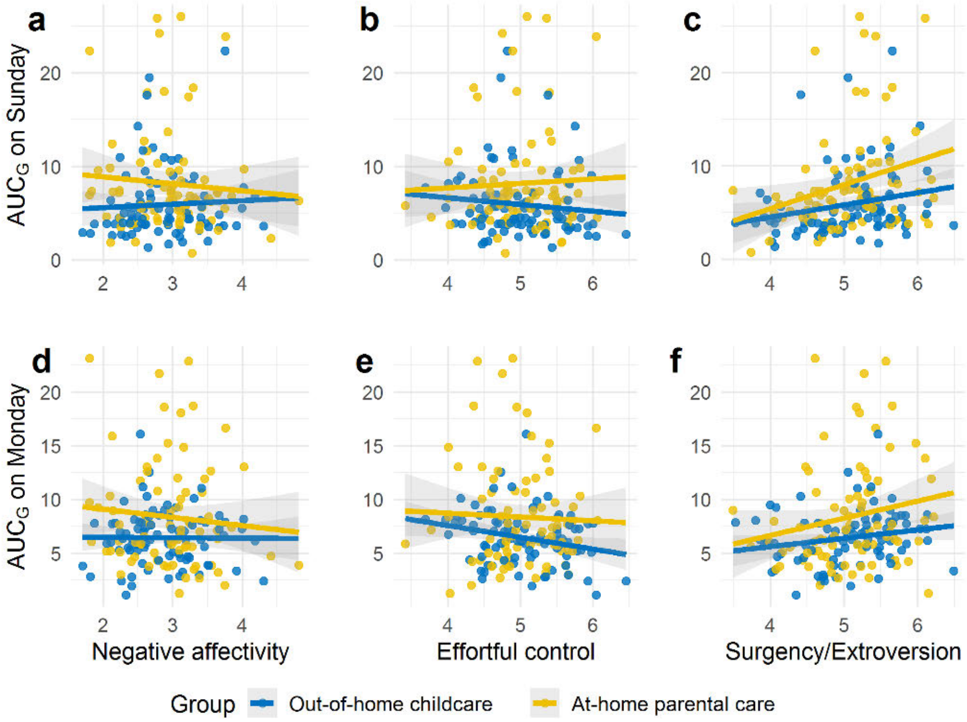

Figure 8. Associations between temperament variables, i.e., negative affectivity, effortful control, surgency/extroversion and total diurnal cortisol AUC_G on Sunday and Monday in both childcare groups.

5.3 Developmental change from 2 to 3.5 years of age (Study III)

Study III was built on our earlier investigations at the child age of 2 years. In the current study, we aimed to extend our past findings by following up the population to the age of 3.5 years and investigating whether the associations between childcare group, temperament and diurnal cortisol retain or change as the children develop.

The results suggest that the overall cortisol levels in both childcare groups decreased along with the child age from 2 to 3.5 years, which is in line with the prior study of Simons et al. (2015) showing that total diurnal cortisol production during the day typically decreases as the children develop. However, in contrast to our earlier findings, there was no significant difference in total diurnal cortisol production between the out-of-home childcare and at-home parental care group (0.97, 95% CI [-0.75; 2.69], p = .27) at the age of 3.5 years. As the result was different from the earlier study at 2 years of age, a post-hoc analysis was conducted to test whether the association was moderated by child age. No significant moderation by age was found (-0.24 [-1.11; 0.64], p = .60).

There were also no significant differences in total diurnal cortisol production between measurement days, i.e., Sunday and Monday, within the groups with at-home parental care being 0.59 [-0.73; 1.91], p = .38 and out-of-home childcare being 0.61 [-0.12; 1.35], p = .10.

However, in line with our earlier findings at the age of 2 years, the afternoon (i.e., 7 h 10 min after awakening) cortisol levels were still 40% ([10%; 79%], p = .007) higher in the out-of-home childcare group during the out-of-home childcare day when compared to their home day (Figure 9 from Original Publication III). In the at-home parental care group, no differences between the measurement days were found (-1% [-32%; 46%], p = .98).

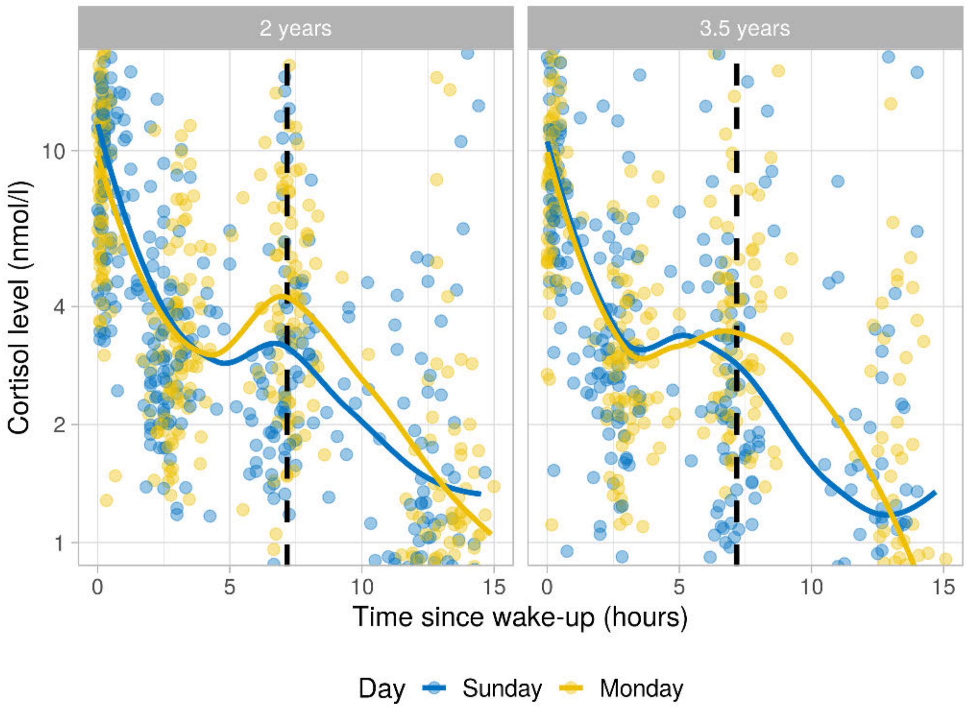

Figure 9. The observed cortisol values (values below 0.90 nmol/l or above 18 nmol/l are not shown) and the diurnal cortisol profiles (based on LOESS regression) in the out-of-home childcare group during their home day (Sunday) and childcare day (Monday) at the age of 2 and 3.5 years. Note, the afternoon naps were controlled for in the statistical models but not in the figure.

Finally, in contrast to our earlier findings at the age of 2 years, there was no significant association between temperamental surgency and total diurnal cortisol production at the age of 3.5 years (0.30 [-0.65; 1.25], p = .54) (Figure 10 from Original Publication III). Post-hoc analysis of the interaction between child age and temperamental surgency further indicated that the association between surgency and cortisol production indeed decreased along with the child age (-1.24 [-2.09; -0.384], p = .004).

Neither Effortful control (0.25 [-0.85; 1.35], p = .66) nor negative affectivity (-0.67 [-1.72; 0.38], p = .21) were related to total diurnal cortisol production at the child's age of 3.5 years.

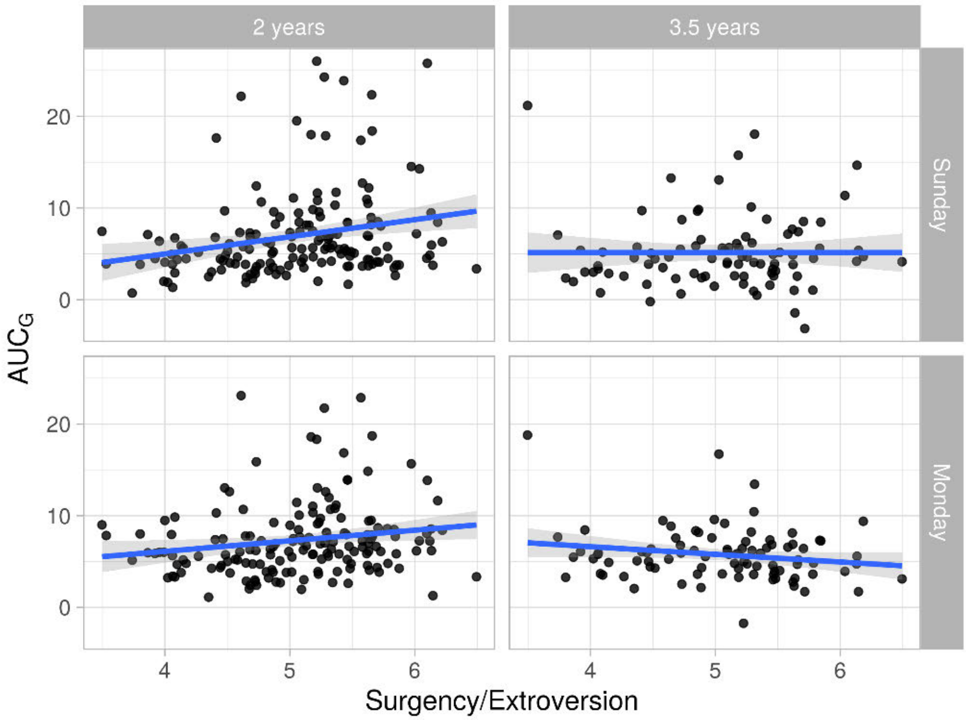

Figure 10. Associations between temperamental surgency and total diurnal cortisol production in the whole study population at the age of 2 and 3.5 years on both measurement days (Sunday and Monday).

5.4 Summary of the results

The results of the current study suggested that at the age of 2 years, the children presented with higher cortisol levels and larger cortisol AUC_G in the at-home parental care when compared to an out-of-home childcare setting. However, these differences between the groups no longer appeared, as the children developed and reached the age of 3.5 years. Additionally, when investigating the diurnal cortisol profiles and slopes in more detail, the results suggested higher afternoon cortisol levels in out-of-home childcare groups during their childcare day when compared to days spent at home. This difference appeared both at the age of 2 and 3.5 years old.

The results further indicate that temperamental surgency/extroversion was associated with higher total diurnal cortisol production in the whole study population at 2 years of age. However, child temperament did not moderate the associations between childcare context and cortisol production. Instead, the association between surgency and cortisol production was similar across the childcare settings. The results further suggest that the strength of the association between surgency and diurnal cortisol production decreased significantly along with the child age, and the association no longer appeared at the age of 3.5 years.

Finally, there was no evidence that the temperament traits negative affectivity and effortful control were related to total diurnal cortisol production.

6 Discussion

In this study, we compared diurnal cortisol levels and total diurnal cortisol production in children participating in out-of-home, center-based childcare and in children who were cared for at home at the age of 2 and 3.5 years. We also examined whether child temperament characteristics (i.e., surgency/extroversion, negative affectivity or effortful control) would be related to total diurnal cortisol production in children. We further investigated whether child temperament would moderate the association between childcare context and diurnal cortisol production. Finally, we investigated whether child age would moderate the association between temperamental surgency and child total diurnal cortisol production.

6.1 Comparison between different childcare settings

Our results in Study I suggested that the shapes of the diurnal cortisol profiles were similar in both childcare groups (i.e., out-of-home childcare or at-home parental care). The cortisol levels followed a typical diurnal secretion profile (Gunnar & Quevedo, 2007), where the cortisol levels are highest approximately 30–45 minutes after waking up in the morning and then decline towards the evening being the lowest before going to sleep (Figure 6). However, at the age of 2 years, the overall cortisol levels and also the total diurnal cortisol production were higher in children who were cared for at home when compared to the children participating out-of-home childcare. This result was unexpected, as we hypothesized that the overall cortisol levels would be higher in children attending out-of-home childcare in comparison with the children cared for at home. This assumption was based on the prior research showing elevated cortisol levels in children during the out-of-home childcare day in contrast to their home days (Geoffroy et al., 2006; Vermeer & van IJzendoorn, 2006). In the out-of-home childcare, the young children need to cope with parental separation and interact with multiple adults and peers, which are suggested to be stressors affecting child cortisol levels in non-parental out-of-home childcare (Legendre, 2003; Vermeer & Groeneveld, 2017). However, earlier studies have not included a comparison group of children, who were cared for at home and who had no prior experience of out-of-home childcare. Instead, the same children have been investigated during their childcare day and home day. Nonetheless, different childcare settings may have distinct effects on child stress regulation during early childhood.

There might be several possible explanations for the differences in overall cortisol levels between the groups in our current study. To begin with, there was more interindividual variation in the cortisol levels between children in the at-home parental care group than in children participating in out-of-home childcare. This might derive from the larger variance in the daily rhythms, such as sleeping, awakening and mealtimes, in the children who were cared for at home (Table 1). Children participating in out-of-home childcare typically follow a similar day-to-day schedule, which increases predictability as a consequence of habituation to the childcare routines and may thus affect child stress regulation. Earlier research suggests that repeated exposure to similar stressors causes habituation and is associated with decreased HPA axis responses (Barnum et al., 2007).

Also, out-of-home childcare attendance is suggested to benefit children, who have several risk factors in their rearing environment (van Huizen & Plantenga, 2018). However, we were not able to assess the quality of care in the at-home parental care in such a detail that could have shed light on the possible associations between caregiving and child stress regulation. Prior research suggests that maternal sensitivity to a child's needs and positive interaction support child stress regulation (Gunnar & Donzella, 2002; Kertes et al., 2009; Tarullo et al., 2017). Maternal depression (Apter-Levi et al., 2016) and anxiety (Simons et al., 2015), in turn, have a strong effect on parenting practices and thereby have a negative influence on child well-being. Children of mothers with depression or anxiety have shown dysregulation of HPA axis functioning (Apter-Levi et al., 2016; Simons et al., 2015). Prior research has also shown that out-of-home childcare attendance may benefit particularly those children who have an adverse home environment (Berry et al., 2014). Out-of-home childcare attendance may also help disadvantaged children to better regulate their emotions and stress responses (Berry et al., 2014; Chryssanthopoulou et al., 2005; Eckstein-Madry et al., 2020). Nevertheless, in this study, we were not able to assess caregiver sensitivity or address possible selection bias between these two groups of children with different childcare contexts.

It is possible that there were some differences between families, who chose out-of-home childcare and those who cared for their children at home at that specific age. However, social selection in childcare attendance in Finland is probably not as high as in many other countries, where the attendance is only possible for higher SES families. In Finland, all the children under school age have a subjective right to participate in Early Childhoohd Education and Care (ECEC) regardless of their parent's employment status (Minedu, 2017). Legislation determines the group sizes in childcare centers and the personnels' education qualities both in public and private childcare units. The quality of ECEC has been considered very high both by parents and by childcare teachers (Hujala et al., 2012). Nevertheless, the enrolment rate in out-of-home childcare in Finland is lower than the OECD average and lower than in other Nordic countries. One reason for the higher home-care rate may be in the home-care allowance, which is rather long in duration and enables parents to care for their children at home until the child's third birthday (OECD, 2020). There is also a tendency for some parents to prefer home care instead of selecting ECEC. In some of the public discussions, the home-care- or family-based childcare has been considered better options for the youngest children. Political discussions, in turn, emphasize parental freedom to choose between different childcare options (Hiilamo, 2004). However, prior findings by Närvi et al., (2020) indicate that there are high socioeconomic differences between mothers, who are returning to work earlier to those who are caring for their children at home for a longer period in Finland. Mothers with a lower employment status and lower education usually take advantage of the government's granted home care allowance for a longer period than those with a higher education (Närvi et al., 2020). This is in line with an earlier study by Wahler et al. (2017) suggesting that mothers with a higher education are more probably taking their children to out-of-home childcare instead of having parental home care. We also noticed in our study sample that the maternal education at the child age of 2 years was higher in the out-of-home childcare group when compared to the at-home parental care group. However, maternal education did not explain the differences in cortisol levels between the groups in the statistical models.

Moreover, children who were cared for at home were slightly younger than children participating in out-of-home childcare in our study sample. A child's age was controlled for in our statistical models, and the age did not explain the differences in cortisol levels between the groups. Nevertheless, a child's HPA axis functioning continues to mature during the early childhood years, and the normal variation in that age group is large (Watamura et al., 2004). Hence, it is also possible that larger interindividual variation and higher cortisol levels in children cared for at home were a part of normal responses to environmental influences in the home-care environment and not necessarily an indication of higher adverse stress levels.

This conclusion was supported by our later findings in the same study population at the age of 3.5 years. The results indicated that there was no longer a difference in diurnal cortisol output between children participating in out-of-home childcare and children who were cared for at home. That is, the differences between the childcare groups did not appear as the children grew older. However, the group difference was not moderated by the child age. It should be noted though that the group size of the children cared for at home was rather small at the age of 3.5 to show possible differences between the groups or moderating role of the child age. Most children in that group had started to participate in out-of-home childcare until 3.5 years of age and were no longer eligible in the second phase of this study.

Finally, it should be noted that, although there were statistically significant differences in cortisol levels between the groups at the age of 2, the absolute differences in median cortisol values (nmol/l) were rather small. Also, the diurnal cortisol profiles followed a typical circadian rhythm, where the levels are highest soon after waking up in the morning and then decline towards the evening (Gunnar & Quevedo, 2007). Importantly, we did not observe the flat or elevated diurnal cortisol slopes as the group level, instead, the cortisol levels declined towards the evening indicating a typical HPA axis functioning in children. However, we do not have a complete understanding about what kind of HPA axis activity across childhood contributes to most optimal or non-optimal developmental outcomes. The dysregulation of HPA axis functioning may appear in hyporeactivity or hyperactivity of the stress regulation systems or inability to return in balance after a stressful experience (Fairchild et al., 2018; Guerry & Hastings, 2011; Nerderhof et al., 2015). It is possible that the HPA axis functioning may change, when it is exposed to a chronic or high stress load. On the other hand, it is also plausible that the pre-existing differences in HPA axis functioning make some people more susceptible to environmental influences (Guerry & Hastings, 2011). Hence, more research of the psychological and physical mechanisms, which form the basis for vulnerability or resilience to the stress, is needed (Lupien et al., 2009).

6.2 Child stress regulation in the out-of-home, center-based childcare context

Prior research indicates that children present with higher cortisol levels in out-of-home childcare, when compared to days spent at home. Out-of-home childcare attendance is associated with higher cortisol levels in particular from mid-morning to the mid-afternoon hours, when compared to decreasing cortisol levels at home (Drugli et al., 2017; Groeneveld et al., 2010; Li & Shen, 2008; Ouellet-Morin et al., 2010; Sumner et al., 2010; Watamura et al., 2009). This is in line with our results in Study I that showed significantly higher afternoon cortisol levels in children participating in out-of-home childcare during their childcare day when compared to their home day. The afternoon cortisol rise was also observed in children, who were cared for at home, but there were no significant differences between the measurement days, i.e., Sunday and Monday.

Children at toddler age normally take naps that lead to a cortisol rise in the afternoon. The presence of the daytime naps influences the diurnal cortisol profile, which however, change and become more like in adults, as the children develop and cease napping (Tribble et al., 2015). Most children in our study sample took naps, which naturally modified the shape of the diurnal cortisol profile (Figure 7). The cortisol rise was most prominent, if the saliva samples were taken between 15 and

60 minutes after awakening from the naps. The higher afternoon cortisol levels in the out-of-home childcare setting during the childcare day were observed both at the child's age of 2 and 3.5 years old. Furthermore, an overall cortisol rise after the naps was more pronounced at the age of 2 when compared to the age of 3.5 years. This is in line with the earlier reports of Watamura et al. (2004) suggesting that the length of the naps influences the afternoon cortisol rise in children. That is, a shorter duration of a nap has been related to lower cortisol rise in children from the mid-morning to the mid-afternoon hours. We did not have information about the length of the naps, but it is possible that children at older ages in our study sample napped for a shorter duration that influenced the post-nap cortisol rise. However, the elevated afternoon cortisol levels in our study sample were not completely explained by the afternoon naps as the napping was controlled for in our statistical models. Hence, it is possible that there are other factors than napping also affecting child afternoon cortisol levels in out-of-home childcare regardless of the child age (Watamura et al., 2002).

The results raise questions about whether the afternoon hours are particularly demanding for children participating in out-of-home, center-based childcare. One explanation could reside in the group size or peer group dynamics, which differ remarkably between the center-based childcare and the home environment. However, prior findings of Gunnar et al. (2010) indicated that even in family-based childcare, with smaller groups, the cortisol levels increased from mid-morning to mid-afternoon, while at home, the levels remained relatively low. We did not find any associations between group size and cortisol output in our study sample either, which might derive from the highly regulated ECEC in Finland and legislation that determines the group sizes and child-to-caregiver ratios. Thus, there were only little variation in group sizes between the childcare centers.

Hence, a more plausible explanation for the afternoon cortisol rise may be in the peer relations and the group dynamics. Cooperation skills develop along with child age (Endedijk et al., 2015), and children are beginning to have a more organized cooperation with peers at that specific age period (Denham et al., 2009). However, social competence and emotion regulation are just developing in the toddler age (Denham et al., 2009), and improved regulation skills may gradually reduce the conflicts that children are involved in with their peer relations (Watamura et al., 2004). On the other hand, group-based childcare settings are also important structured environments for learning social skills (Larose et al., 2020). Prior findings suggest that toddlers, who managed to play more with peers, had lower diurnal cortisol levels than those who were less involved in play during the out-of-home childcare day (Watamura et al., 2003). Hence, a peer group context is very important for children and has many positive effects on child development. Peer acceptance constitutes protective factors against externalizing problems and rejection. Adverse influences may occur in those children who do not manage to engage in peer play or are rejected from other children (Hay et al., 2004).

Another plausible explanation for the higher afternoon cortisol levels in out-of-home childcare may derive from the separation from parents. Children participating in full-time childcare in Finland usually spend rather long days in childcare centers, as their parents are working. Secure attachment to the mother has helped a child to adapt into a new out-of-home childcare environment and creates a buffering effect for the rising cortisol levels, when the parent was present (Ahnert et al., 2004; Gunnar & Hostinar, 2015). However, when the mother was no longer present, the cortisol levels rose similarly as in those children with an insecure attachment to a primary caregiver (Ahnert et al., 2004). Earlier research also suggests that children participating in full-day and full-time, out-of-home childcare presented with higher cortisol levels during a childcare day when compared to half-day or part-time enrolment (Lumian et al., 2016). This is in line with our findings in which the robust analysis indicated that children participating in full-time childcare, 20 days per month, had higher cortisol levels than children having part-time care at a maximum of 16 days per month. Also, long hours per day in out-of-home childcare have been associated with elevated afternoon cortisol levels in children (Drugli et al., 2017). A prior study also suggests that children have shown higher cortisol levels even for weeks after starting in a new out-of-home childcare setting (Bernard et al., 2015). However, the length of the childcare attendance was not related to cortisol levels in our study sample. This may derive from the fact that most children had participated in out-of-home childcare already for months and were adjusted for the childcare routines and were no longer beginners.

Finally, the quality of care is one of the most important factors affecting child stress regulation regardless of the childcare setting. Earlier studies have shown that a low quality of care was associated with an alteration in stress regulation and higher cortisol levels both in home-based and in center-based childcare as well as in preschool settings (Dettling et al., 2000; Groeneveld et al., 2010; Gunnar et al., 2010; Lisonbee et al., 2008; Sajaniemi et al., 2014; Sims et al., 2005, 2006). A secure attachment to caregivers in out-of-home childcare has been associated with decreasing cortisol levels in children during the day (Badanes et al., 2012). However, we were not able to include the quality indicators or attachment measurements in this study. Hence, future studies should take into account the quality aspects in order to investigate their moderating effect on the associations between the childcare context and stress regulation.

In summary, prior studies have repeatedly observed higher cortisol levels in children participating in out-of-home childcare during their childcare day. Higher afternoon cortisol levels in our study sample were partly explained by the afternoon naps, but there are most likely other factors also affecting child cortisol levels in an out-of-home childcare setting. The childcare teachers and caregivers should consider this when implementing schedules and planning ECEC programs. The afternoon hours may be particularly demanding for the youngest children. Future studies should consider both environmental and child individual characteristics, which modify the stress responses in out-of-home childcare settings. It is important to recognize those children who are in higher risk for showing elevated cortisol levels. It is also important to pay special attention to the adaptation period in a new childcare setting and ensure parent's and caregiver's ability to support child in at a new developmental stage.

6.3 Associations between child temperament and total diurnal cortisol production

Prior studies suggest that child temperament may play an important role in early childhood stress regulation and sensitivity to environmental influences (Donzella et al., 2000; Geoffroy et al., 2006; Talge et al., 2008; Watamura et al., 2004). We hypothesized in our Study II that children with higher negative affectivity, higher surgency/extroversion and lower effortful control would have higher total diurnal cortisol production in out-of-home childcare when compared to the home setting. This was based on the assumption that out-of-home, center-based childcare context would set more challenges for young children with these temperament characteristics.

The results of the present study suggest that temperamental surgency, an aspect of positive affectivity PA, was associated with higher total diurnal cortisol production in the whole study population, when the childcare group and the measurement day were controlled for in the statistical models. The child negative affectivity and effortful control were not related to diurnal cortisol output. We further investigated whether child temperament would moderate the associations between cortisol production and childcare context. In contrast to our hypothesis, we found no evidence that temperament would play a moderating role in the association between childcare and cortisol production. Instead, the associations between temperament characteristics and cortisol output were similar across the childcare settings.

Our results are partially in line with the earlier findings suggesting that higher surgency would be associated with higher cortisol levels in children. However, earlier studies have mainly focused on the transitions to novel situations or stress reactivity tests in laboratory circumstances. For instance, in studies focusing on the transition from preschool to elementary school or the beginning of a new school year, temperament traits of higher surgency or lower effortful control were related to higher cortisol levels in children (Davis et al., 1999; Hall & Lindorff, 2017; Turner-Cobb et al., 2008). This is plausible, because inter-individual differences in temperament may be associated with the rate of adaptation and social approach/inhibition, which in turn, may mediate the stress responses to context. Furthermore, children higher in surgency have shown higher stress reactivity in the laboratory test situation including competition and challenges (Donzella et al., 2000). Thus, it is possible that children higher in surgency are physiologically more reactive to environmental stimuli. The biological basis for PA may determine central nervous system reactivity and behavior in particular for children at that age period.

Also, children higher in surgency are generally sociable, and they have more interaction with peers when compared, for instance, to children higher in negative affectivity (Endedijk et al., 2015). However, children higher in surgency are also at a higher risk for impulsivity and enhanced approach behaviors, which may lead to externalizing problems in a peer group context (Berdan et al., 2008; Morales et al., 2015; Stifter et al., 2008). Although children higher in surgency are sociable, they may interact in a way that affects peer rejection and coflicts for some children (Dollar & Stifter, 2012). Thus, it is possible that higher surgency was related to higher cortisol production through behavioral patterns also in our study sample.

Contrary to our hypothesis, effortful control, which is a self-regulatory aspect of temperament, was not related to child cortisol production. Effortful control is shown to increase during the childhood years and is an important predictor of well-being and social competence as well as ability to regulate stress responses (Denham et al., 2003; Denham & Holt, 1993). Earlier findings by Watamura et al. (2004) indicated that children with less mature effortful control presented with higher total diurnal cortisol production in their daily activities. This may derive from the conflicts and challenges that children with lower effortful control, and thus with lower self-regulation capacity, frequently encounter.

A prior study by Gunnar et al. (2003) showed that the combination of high surgency and low effortful control was related to higher cortisol levels in preschool aged children. Those children had more aggressive behavior and rejection in their peer group contexts. That is, excuberant behavior and a weak ability to regulate emotions may lead to externalizing problems and a higher stress load in children (Gunnar et al., 2003). Unfortunately, our study did not have enough power to investigate the combination of high surgency and low effortful control and its relation to child stress regulation. However, future studies should consider this option, as it may play an important role in early childhood stress regulation and well-being.

Finally, child negative affectivity (NA) was not associated with higher total diurnal cortisol production, and it did not moderate the association between the childcare setting and cortisol output either. This is in contrast with earlier studies that have shown that, in particular, fearfulness and behavioral inhibition have been associated with higher cortisol levels and stronger stress reactivity (Kagan et al., 1987; Talge et al., 2008). Also, NA and specifically social fearfulness and shyness have been associated with higher cortisol levels in children participating in out-of-home childcare and preschool (Albers et al., 2016; Dettling et al., 2000; Watamura et al., 2002). However, we found no evidence for the associations between NA and diurnal cortisol production in the out-of-home childcare context. One explanation for this may lie in the rather high educational level of the childcare teachers and other personnel in out-of-home childcare centers in Finland. Professional quality may help the childcare personnel to respond to the child's needs and diminish the influence of out-of-home childcare on child stress regulaton in children with different temperament characteristics.

Nevertheless, it is also possible that children higher in NA are more reactive to acute stressors that were not measured in this study. We focused on the total diurnal cortisol production AUC_G that describes the overall activity of the HPA axis during a day, but discards the information of the diurnal cortisol slopes or cortisol responses to specific acute stressors (Rotenberg et al., 2012). NA and fearfulness have been ssociated with higher stress reactivity in the laboratory test situations (Talge et al., 2008), and thus, future studies should test stress reactivity and recovery rates also in authentic situations both in out-of-home childcare and in at-home parental care settings.

Also, the theoretical framework of individual differences to environmental sensitivity (Belsky 2005) could explain the associations between cortisol production and a childcare setting. According to the theory, the children are, for temperamental and genetic reasons, differentially susceptible to environmental influences. In particular, the temperament trait of NA may be one marker of susceptibility to both adverse environmental effects and, on the other hand, beneficial effects of supportive rearing (Belsky, 2005; Groeneveld et al., 2012; Slagt et al., 2016). However, we were not able to study the quality of care in such a detail that could have shown its moderation effect in the association between temperament and cortisol production. Hence, the following studies should consider environmental factors in more detail, because they may have a different effect on children with different temperament characteristics.

6.4 Developmental change in the association between surgency and total diurnal cortisol production

At the child age of 2 years, we found a sound association between temperamental surgency and total diurnal cortisol production in the whole study population. We expected that the relation between surgency and cortisol production would be associated with such basal physiological attunement that is shown as higher durnal cortisol production in young children. We also assumed that this connection would remain as the children develop. That is, children higher in surgency could be physiologically more reactive to environmental influences independent of the childcare context. However, in contrast to the hypotheses, surgency was not associated with total diurnal cortisol production at the child age of 3.5 years in Study III. Indeed, the association between surgency and cortisol production diminished significantly along with the child age from 2 to 3.5 years.

The surgency refers to a temperamental dimension of positive affectivity, which consists of a tendency to approach behavior, a high activity level and sensation seeking (Rothbart & Bates, 2006). One explanation for the association between surgency and cortisol production at a younger age may lie in the socioemotional development of the child. Toddlerhood is an important period for the development of social and emotional competence and the ability to interact in a social environment such as in peer-group context (Brownell & Kopp, 2007; Cole et al., 2018). Good effortful control and the ability to regulate emotions predict a better interaction with peers and adults and may also decrease the stress load in social situations (Denham et al., 2003; Denham & Holt, 1993). Earlier findings by Watamura et al. (2004) support this conclusion of regulation capacity and its role in child stress load. That is, the HPA axis maturation may be closely related to the behavioral development of the child (Watamura et al., 2004). Hence, it is plausible that children higher in surgency in our study sample were physiologically more reactive to environmental stimuli, when they were younger. However, the role of surgency for cortisol production was less remarkable as the children developed. The development of effortful control (Denham et al., 2003) and inhibition (Best & Miller, 2010) may weaken the negative effects of surgency, as the children are able to better control their emotions.

However, there are very few studies that would have investigated the role of temperament in child stress regulation during a longer period across childhood years. Earlier studies have shown that the diurnal cortisol production typically decreases along with the child age (Simons et al., 2015). Also, cortisol output in an out-of-home childcare context is suggested to change as the children develop (Ouellet-Morin et al., 2010; Watamura et al., 2003). Our results indicated that there might be age dependency also in the associations between temperament and child diurnal cortisol production independent of the context. Hence, more longitudinal research is needed to understand the periods of sensitivity in the early childhood stress regulation in children with different temperament characteristics.

6.5 Strengths, limitations and future directions

There are several strengths in this study such as its prospective study design, a rather large sample size, several saliva cortisol measures during a day; the comprehensive background information of the participants and the accurate statistical analyses, which considered awakening and sleeping times as well as different daily rhythms of the children. Furthermore, we were able to include a comparison group of the children, who were cared for at home, which made this study design rather unique in the field. Previous research concerning the child stress regulation in an out-of-home childcare environment have mainly examined the intraindividual differences between the home day and childcare day. However, there is a notable lack of research that include the at-home parental care comparison group of the children, who have not yet participated in non-parental, out-of-home childcare. Also, children with different temperament characteristics have shown different kind of responses to environmental influences. Hence, it is important to consider whether these responses are more related to physiological reactivity or to specific environmental characteristics such as the childcare setting. These questions are difficult to answer without having a comparison group of children with distinct childcare settings.

Despite the many strengths in this study, there are limitations that should be noted. To begin with, the saliva samples were collected only during two days per group, i.e., Sunday and Monday, per age point. Cortisol levels may vary from day to day, and normal variation in this age group is large (Watamura et al., 2004). Having more consecutive collection days per context could have increased the reliability of the study and diminished the possible effect of the variation between the measurement days. However, we were faced with the challenges of ensuring optimal measurements versus the resources and maximal efforts that may have been required from the participants. The parents were asked to collect four saliva samples from toddlers during two consecutive days at two age points and simultaneously report the child's daily rhythms and activities during collection days. The out-of-home childcare personnel collected the samples in the out-of-home childcare and reported the corresponding information during the childcare days. More collection days could have caused a drop-out of the participants or influence the quality of the samples, as the study protocol was already rather demanding for the parents and childcare personnel. However, we have collected four saliva measurements during a day at two consecutive days per age point, which is more than many other studies in out-of-home childcare environments. Additionally, we calculated the correlations between measurement days, i.e., Sunday and Monday, in Study II to examine intraindividual variation between the measurement days. The results indicated a strong correlation between the days. The correlation was higher in the at-home parental care group indicating more similar measurement days, i.e., Sunday and Monday, in children, who were cared for at home when compared to children participating out-of-home childcare.

Furthermore, the computation of AUC is suggested to be the most stable cortisol indicator in comparison with the diurnal slope, cortisol awakening response or single sample measures. The AUC measures are recommended, when the diurnal measurements are limited to only one day per context (Ross et al., 2014; Rotenberg et al., 2012). We examined both total diurnal cortisol production AUC_G and diurnal cortisol slopes in our statistical models, which probably increases the validity of our study. However, in the future, it would be important to implement childcare studies with more consecutive measurement days in a comprehensive study population. A larger sample size would have enabled a more complex interaction analysis using multiple variables.

Among the limitations is also the fact that we were not able to study the quality of care in out-of-home childcare centers or detailed family circumstances of the children who were cared for at home. We collected the basic information, such as the daily schedules, sleeping, awakenings and mealtimes. However, a more detailed information of the life events and circumstances both at home and in out-of-home childcare could have increased our understanding of their potential influence on child stress regulation. Furthermore, it would have been important to resolve parental motivations and reasons behind the choices to take care of their child at home or alternatively select out-of-home childcare.

It is well known that early life environment and in particular primary caregiving, attachment and maternal well-being have a strong effect on the child's HPA axis functioning (Badanes et al., 2012; Gunnar & Quevedo, 2007; Gunnar & Cheatham, 2003). Furthermore, the peer relations are important factors that may potentially affect the cortisol levels in out-of-home childcare. Future studies should thus consider the quality aspects, peer relations and the child attachment to caregivers both at home and in out-of-home childcare. It would also be important to include theoretical perspectives of different susceptibility (Belsky & Pluess, 2012) or goodness-of-fit (Thomas & Chess, 1989) in particular to the studies concerning temperament dimensions.

Finally, most of the children in the at-home parental care group had started to participate in out-of-home childcare by the age of 3.5 years, which was our second measurement point in Study III. Therefore, the sample size of that group was rather small, and the results should be interpreted with caution. Changing the measurement age to a maximum of 3 years could have decreased the drop-out and help keep that group larger in the second phase of the study. However, that would have required rather large changes to our original research plan and was not possible in the context of the current study. Nevertheless, future studies should replicate the same type of research with a bigger sample size in order to shed more light on the possible enduring or disappearing associations between the childcare context and child stress regulation.

7 Conclusions

The comparison between out-of-home childcare and at-home parental care indicated some differences in 2-year-old toddler's diurnal cortisol output in these two different childcare settings. In contrast to our expectations, the children who were cared for at home presented with higher overall cortisol levels in comparison with the children participating in out-of-home childcare. Although the differences between the groups were modest, it is important to consider possible reasons for the distinct cortisol production and their future implications. There was a larger variation in children's daily rhythms in the at-home parental when compared to the children participating in out-of-home childcare. A regular day-to-day schedule, which is typical in out-of-home childcare, increases predictability and may thus have a positive effect on child stress regulation (Barnum et al., 2007). Out-of-home childcare attendance with Early Childhood Education and Care programs is also suggested to benefit children who have risk factors in their home rearing environment (van Huizen & Plantenga, 2018). Nevertheless, we were not able to assess the quality of care in the at-home parental care in such a detail that could have shed light on the possible associations between caregiving and child stress regulation. However, it is important to recognize the children who are at a higher risk for elevated stress levels in their home rearing environment and would benefit the ECEC. Out-of-home childcare participation could be more strongly recommended for the children who need more support.

When assessing only the out-of-home childcare group as a whole, the results showed significantly higher afternoon cortisol levels in children during the childcare day when compared to their home day. The afternoon hours may be particularly demanding or stimulating for the children in out-of-home childcare independent of the child age. Peer relations and interaction quality within the childcare groups are also important factors for child stress regulation and well-being (Badanes et al., 2012; Endedijk et al., 2015; Gunnar et al., 2003). Thus, the childcare personnel should consider these aspects when implementing schedules and planning ECEC programs. It is also important that the number of personnel is adequate and allows for individual support for the child. Prior findings also suggest that full-time childcare and longer daily hours in out-of-home childcare are physiologically more demanding than part-time care and promotes HPA axis activation in children (Drugli et al., 2017; Lumian et al., 2016). Many children in Finland spend rather long days in out-of-home childcare. Hence, it could be appropriate for parents to arrange shorter days and part-time care for the youngest children, if possible. Also, the adaptation period into a new childcare context and separation from parents may be a particularly stressful challenge for young children (Ahnert et al., 2004; Gunnar & Hostinar, 2015). Hence, the parents and caregivers should pay particular attention to that period and give the child enough time to adjust to the non-parental, out-of-home childcare.

Finally, a child's individual characteristics, such as temperament, is suggested to play a role in early life stress regulation and sensitivity to environmental influences in particular to out-of-home childcare settings (Albers et al., 2016; Geoffroy et al., 2006; Phillips et al., 2011; Watamura et al., 2003). However, our findings indicated that temperament plays only a minor role in child stress regulation in the out-of-home childcare, at least in the Nordic model of ECEC systems. The associations between temperament characteristics and child diurnal cortisol production appeared similarly both in out-of-home childcare and in at-home parental care settings. At the child age of 2 years, temperamental surgency was related to the higher diurnal cortisol production in the whole study population, but that association diminished along with the child age from 2 to 3.5 years old. It is possible that child socioemotional development (Watamura et al., 2004) decreases the role of surgency for diurnal cortisol production as the children develop. However, there are very few studies that would have investigated the role of temperament for child stress regulation in a longer period across childhood years. In particular, the moderating role of the associations between high surgency and low effortful control should be investigated in more detailed. Hence, more longitudinal research is needed to understand the periods of sensitivity in early childhood stress regulation in children with different temperament characteristics. Future studies should also consider other important factors, such as, peer relations, quality of care and socioemotional development and their associations with child stress regulation in different childcare settings.

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