Doctoral theses at NTNU, 2023:323

Kathrin Nystad

Toddlers' childcare stress across context and time

A cross-sectional and longitudinal investigation of children's cortisol levels during transition, in childcare, and at home - and their relation to childcare, child, and family factors

Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Medicine and Health Sciences
Department of Mental Health

NTNU
Norwegian University of
Science and Technology

Kathrin Nystad

Toddlers' childcare stress across context and time

A cross-sectional and longitudinal investigation of children's cortisol levels during transition, in childcare, and at home - and their relation to childcare, child, and family factors

Thesis for the Degree of Philosophiae Doctor

Trondheim, October 2023

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Mental Health

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the Degree of Philosophiae Doctor

Faculty of Medicine and Health Sciences
Department of Mental Health

ISBN 978-82-326-7346-9 (printed ver.)
ISBN 978-82-326-7345-2 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (online ver.)

Doctoral theses at NTNU, 2023:323

Printed by NTNU Grafisk senter

Sammendrag

Små barns barnehagestress på tvers av kontekst og tid

Hjernen er under rask utvikling i barns første leveår og i denne perioden dannes et livslangt helsegrunnlag. I Norge går nesten alle ett- og toåringer i barnehagen og det er derfor viktig å skaffe til veie mer kunnskap om hvordan de har det i barnehagen. Det er funnet at små barn har et forhøyet nivå av stresshormonet kortisol i barnehagen. Dette er et fenomen vi trenger mer forskning på. Det er for eksempel uklart hvilken type stress dette er, i hvilken grad den er begrenset til barnehagekonteksten, og hvordan den utvikler seg over tid. Kortisol, målt i spytt, er en anerkjent indikator på opplevd stress. Å undersøke små barns kortisolnivå kan bidra til økt forståelse av hvordan de opplever barnehagen, hvilke barn som er mest utsatt for barnehagestress og sammenhenger mellom barnehagekvalitet og stress. I studiene som inngår i denne avhandlingen er små barns kortisolnivå undersøkt, både på tvers av kontekster (i barnehagen og hjemme) og over tid (fra tilvenningen og gjennom et helt barnehageår), samt at det er sett på hvordan endringer av stressnivå henger sammen med kjennetegn ved barnet, familien og barnehagen.

For å måle endringer av barnas kortisolnivå, ble det tatt spyttprøver av 320 barn ved til sammen 30 anledninger, om morgen, ettermiddagen og kvelden under tilvenningen til barnehagen og i starten, midten og slutten av et barnehageår. I tillegg ga spørreskjemaer og observasjoner informasjon om blant annet barnas temperament og trivsel i barnehagen, samt barnehagekvalitet. Lineære blandete effektmodeller ble brukt i de statistiske analysene.

Barna hadde et forhøyet stressnivå på alle ettermiddagsmålinger i barnehagen, mens de hadde et lavt stressnivå på kvelden hjemme og både på ettermiddag og kveld på helgedager

hjemme. Funnene indikerer at barna opplevede barnehagen og hjemmet forskjellig og at lav barnehagekvalitet forsterket denne forskjellen: Barna var mer stresset i avdelinger med flere enn tre barn per voksen og i avdelinger som hadde mindre forutsigbare rammer. Under tilvenningen til barnehagen hadde stressnivået et kurvlineært mønster. Barna hadde et lavt stressnivå så lenge foreldrene var til stede sammen med dem i barnehagen, mens de var tydelig stresset den første uken de var adskilt fra foreldrene. Etter cirka en måned i barnehagen hadde stressnivået falt til et lavere nivå, men var fortsatt noe forhøyet. De aller yngste barna hadde et høyere stressnivå ved dette tidspunktet. Barna ble fulgt videre gjennom barnehageåret og i gjennomsnitt så det ut som om de akkumulerte noe stress over tid ved at de viste stigende nivåer av morgen- og kveldskortisol. Dette langsiktige stressmønsteret delte seg i to profiler i henhold til trivsel i barnehagen: Barn med veldig høy trivsel viste tegn til å gradvis falle mer til ro i barnehagen mens barn med lavere trivsel hadde et høyere og stigende stressnivå over tid og så dermed ut til å bli mer stresset gjennom året.

Barnehagekonteksten så ut til å være mer krevende enn hjemmet for barna og dette kan tyde på at barnehagepraksis ikke er ideelt tilpasset til de minste barnas forutsetninger og behov. Våre funn indikerer at en forsvarlig bemanning av minst en voksen per tre barn gjennom hele åpningstiden, stabile og forutsigbare rutiner, rolige ettermiddager, og oppmerksomhet på de aller yngste barna, samt fysisk passive barn, er viktig for barnas stressregulering gjennom barnehageåret. Stressmønsteret som ble observert under tilvenningen peker på hvor viktig foreldres involvering i overgangen fra hjem til barnehage er, og at de aller yngste barn trenger mer tid til å falle til ro i barnehagen. Barna så ut til å ha en forskjellig tilpasning til barnehagen gjennom året, basert på hvordan ansatte vurderte deres trivsel. Mekanismene bak dette funnet er vanskelig å vurdere, men med støtte fra tidligere forskning er det rimelig å påstå at ansatte må se

og inkludere barn som er tilbaketrukne og lett blir oppskaket, samt de som vurderes til å ikke trives, og tilrettelegge for godt samspill mellom voksne og barn og barna imellom.

Det vurderes at det stressnivået som er observert i denne avhandlingen, er moderat og tidsbegrenset, men at det samtidig skjedde noe stressakkumulering hos barna gjennom barnehageåret. Siden stressnivået viste seg å henge sammen med lavere trivsel og lavere barnehagekvalitet, konkluderes det at dette ikke utelukkende representerer såkalt positivt stress. Framtidig forskning bør blant annet undersøke barnehagestress i et langtidsperspektiv nærmere, samt dens effekter, og hvordan dyadisk samspill med voksne kan regulere barnas stressnivå.

Abstract

Toddlerhood is a stage of rapid brain development, during which the foundation for a life-long health trajectory is built. With a large and increasing share of young children attending out-of-home childcare, it is vital to know how they are faring in this context. Toddlers have been observed to have elevated levels of the stress hormone cortisol in childcare and this phenomenon needs to be understood better. It is unclear which type of stress the observed cortisol elevations constitute and whether these elevations are limited to the childcare environment, and how they develop over time. Cortisol is recognized as marker of perceived stress and can give us an indication of children's experience in childcare. Investigating the link between cortisol levels and child and family characteristics as well as childcare quality can help us to determine if there are groups of children who are more prone to stress in childcare and if there are childcare practices which may trigger or alleviate stress. These insights could help us to better adjust childcare practice to toddlers' needs. The studies included in this thesis have measured young children's cortisol levels both cross-sectionally (in childcare and at home) and longitudinally (starting from the transition to childcare and throughout a year in childcare) and examined how stress levels were linked to child, family, and childcare factors.

Saliva samples were collected from 320 children in the morning, afternoon, and evening during the initial transition to childcare and at the beginning, middle, and end of a year in childcare and analyzed for cortisol. Parents and caregivers respectively filled questionnaires on age, gender, temperament, number of siblings, childcare quantity, and maternal education, as well as group size, the number of caregivers and pedagogues among the caregivers, children's well-being in childcare, and organizational flow in group units. In addition, interactional quality was observed. Linear mixed models were applied for statistical analysis.

Children had elevated cortisol levels in the afternoon in childcare on every measurement occasion. However, cortisol levels were distinctly sinking towards the evening and on weekend days at home. The childcare context seemed to be more demanding than the home context for the children in our studies. Lower childcare quality exacerbated this difference: Children had higher cortisol levels in childcare when they were attending group units with more than three children per caregiver and a higher degree of disorganization. Our cross-sectional investigation indicated that childcare practice may have not fully matched to the needs of toddlers. A sufficient caregiver availability, stable and predictable routines, quiet afternoons, and a focus on young and passive children are likely highly important for stress regulation. The longitudinal investigation started from one-year-olds' initial transition to childcare. Cortisol levels followed a curve-linear pattern during transition, with low levels on days with parents present, high levels in the first week separated from parents and comparably lower levels after a month of childcare attendance. Acclimatization days with parents likely offered an important time window to familiarize children with the childcare environment. The youngest children had higher afternoon cortisol levels after a month in childcare and may have needed longer time to settle in at childcare. The investigation continued through a year in childcare, and, on average, it seemed that children accumulated some stress throughout the year as indicated by increasing evening and morning cortisol levels. This longitudinal stress pattern split into two profiles according to children's well-being in childcare: Children with very high well-being showed a decrease in afternoon cortisol elevations while other children had higher overall cortisol levels and increasing morning and evening cortisol levels throughout the year and thus showed more distinct signs of a stress accumulation. Hence, children seemed to follow different patterns of long-term adaption to childcare based on their well-being. The mechanisms behind this finding are difficult to assess,

but with support from previous research, it is reasonable to assert that caregivers must see and include children who are withdrawn or easily upset, as well as those they assess as not thriving.

The observed stress was likely moderate and time-limited, yet there was some long-term stress accumulation. Since stress was associated with lower well-being and aspects of lower childcare quality, it is concluded that cortisol elevations in childcare do not merely represent positive stress. Future research must examine childcare stress in a longitudinal perspective, as well as its effects, and how dyadic interactions with caregivers can regulate children's stress levels.

Navn kandidat: Kathrin Nystad

Institutt: Regionalt kunnskapssenter for barn og unge – Psykisk helse og barnevern (RKBU Midt-Norge), Institutt for psykisk helse, Fakultet for medisin og helsevitenskap, NTNU

Veiledere: May Britt Drugli (NTNU), Ratib Lekhal (UiO)

Finansieringskilde: Norges forskningsråd, program: «BEDREHELSE»

Ovennevnte avhandling er funnet verdig til å forsvares offentlig

for graden PhD i medisin og helsevitenskap.

Disputas finner sted i BS 31, Bevegelsessenteret, NTNU,

fredag 13. oktober 2023, kl. 12.15.

Contents

Acknowledgements

I am incredibly lucky and grateful for the opportunity to go on a PhD journey and would like to express my gratitude to everyone who has supported me along the way.

Firstly, I want to thank the Norwegian Research Council for funding the Thrive by Three study and thereby my research as well as the Department of Mental Health and RKBU at NTNU Trondheim for providing me with a positive and stimulating academic work environment. Thank you to the Thrive by Three team for letting me look behind the scenes of a big and important research project and including me into your work from day one.

This thesis would have not been possible without the guidance of my brilliant supervisors May Britt Drugli and Ratib Lekhal. I want to especially thank May Britt for her encouraging supervision. Your constant support and guidance helped me to improve a lot, and I learned many useful skills under your watch.

I am grateful to my co-authors, Stian Lydersen, Elisabet Solheim Buøen and Håvard Horndalen Tveit for their invaluable feedback and the interesting discussions. A special thank you goes to Stian for statistical guidance. I also want to thank Anne Synnøve Brenne for her support and our inspiring conversations about the youngest children in childcare. A big thank you goes to all my colleagues in the loft for company and encouragement. I also want to thank all childcare employees, parents and children who participated in the labor-intensive data collection of the cortisol studies.

Last but not least, I am grateful to my unconditionally supportive family, Doris Bruckmeier, Manfred Bösch, Christoph Bösch, Angela von Schönberg, Reiner Jäschock, Maria Jäschock, Dieter Jäschock, Turid Johns and Ronald Nystad, and especially to my wonderful and very patient husband Ole Edward Johns Nystad and my lovely son Matthias Nystad.

Trondheim, May 2023
Kathrin Nystad

List of papers

Paper 1: Toddlers' stress during transition to childcare. *Published in The European Early Childhood Education Research Journal (2021)*.

Paper 2: Toddlers' cortisol levels in childcare and at home. S*ubmitted to Early Education and Development.*

Paper 3: Change in toddlers' cortisol activity during a year in childcare. Associations with childcare quality, child temperament, well-being, and maternal education. *Published in The International Journal on the Biology of Stress (2022)*.

Abbreviations

ACTH	Adrenocortical trophic hormone
AVP	Arginine vasopressin
BCa	Bias-corrected and accelerated
CAR	Cortisol awakening response
CLASS	Classroom Assessment Scoring System
CRH	Corticotropin-releasing hormone
CV	Coefficient of variation
DELFIA	Competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection
EAS	Emotionality Activity Shyness Temperament Survey
ECERS-R	Early Childhood Environment Rating Scale Revised
EBS	Emotional and Behavioral Support Scale of CLASS Toddler
ESL	Engaged Support for Learning Scale of CLASS Toddler
GR	Glucocorticoid receptors
HPA	Hypothalamic-pituitary-adrenal axis
IT-CC-HOME	Infant Toddler Child Care Home Observation for Measurement of the Environment inventory
ITERS-R	Infant Toddler Environment Rating Scale Revised
LICW-D	Leiden Inventory for the Child's Well-being in Daycare
LECP	Life in Early Childhood Programs Scale
MR	Mineralocorticoid receptors
NICHD	Eunice Kennedy Shriver National Institute of Child Health and Human Development
NSD	Norwegian Center for Research Data
NTNU	Norwegian University of Science and Technology
OECD	Organization for Economic Co-operation and Development
PVN	Paraventricular nuclei
Q-Q plots	Quantile-quantile plot
REK	Regional Committee for Medical and Health Research Ethics
SAM	Sympathetic-adrenal-medullary system
UNCRC	The United Nations Convention on the Rights of the Child

1 Introduction

1.1 Topic of the thesis

A large and increasing proportion of toddlers (one- and two-year-old children) in the OECD-countries is enrolled in an early childcare arrangement (OECD, 2017). Nearly all toddlers (87%) attend childcare in Norway, and most of them stay for more than eight hours each day (Statistics Norway, 2022e). Since childcare plays such a big role from early on in many children's lives, it is vital to know how toddlers are faring in childcare. Measuring their stress levels may be a way of assessing their experience in childcare (Vermeer & Van Ijzendoorn, 2006).

Toddlerhood is a period characterized by high brain plasticity. Toddlers rapidly develop their emotional and cognitive skills in a constant exchange with their caregivers and their environment. Stable, reciprocal and stimulating relationships and a sense of security are essential for healthy brain development (Center on the Developing Child, 2004b). In this context, childcare attendance holds many potential benefits for children. They can form social bonds, learn and play (Brooker, 2008). Children attending early childcare have been observed to have better cognitive skills and language abilities (Camilli et al., 2010; OECD, 2017) and especially children from risk backgrounds may profit (Cadima et al., 2020; Orri et al., 2021). However, for benefits to arise, childcare quality needs to be sufficiently high (Center on the Developing Child, 2004b; NICHD Early Child Care Research Network, 2005) and adapted to children's individual needs (Phillips et al., 2011).

Gaining an understanding of toddlers' experience of childcare is essential information for adjusting practice to their needs. Researching children's stress levels in childcare may give us insight into their perception of the childcare context (Vermeer & Van Ijzendoorn, 2006).

Researchers have observed elevated levels of the stress hormone cortisol in toddlers in childcare, which means that toddlers seem to be slightly stressed in childcare (Vermeer & Van Ijzendoorn, 2006). Baseline cortisol levels follow a pattern of diurnal decline in humans from approximately late infancy (Watamura et al., 2004). The body releases additional cortisol in demanding situations and rising cortisol levels during the day are recognized as an indicator for social stress (Kirschbaum & Hellhammer, 1994). Hence, measurements of children's cortisol levels have been applied to assess their experience in childcare and thereby to include young children's perspective in quantitative research studies (Vermeer & Van Ijzendoorn, 2006). Finding out how toddlers' childcare stress is linked to context, time, child and family characteristics and childcare quality can aid us in identifying groups of children who are more vulnerable to childcare stress, stress-inducing childcare practice as well tendencies of stress accumulation, and may thereby help us to adjust childcare better to children's needs. However, due to little research, there is not enough knowledge on toddlers' stress in childcare (Vermeer & Groeneveld, 2017). It is still unclear how childcare stress develops during the initial transition between home and childcare and during extended periods of time, and if it spills over into the home context (Ahnert et al., 2022; Engel & Gunnar, 2020). Childcare stress is likely linked to characteristics of the child, family, and childcare practice. However, previous research has produced ambiguous results regarding these relations (Vermeer & Groeneveld, 2017). The aim of the present thesis is to study toddlers' stress in childcare by conducting longitudinal and cross-sectional observations of their cortisol levels in childcare and at home during different stages of a year in childcare (initial transition from home to childcare, cross-sectional in childcare and at home, longitudinal through a year in childcare) and to investigate how cortisol levels are linked to child, family, and childcare factors.

In the following sections, I will discuss the theoretical background and previous research on toddlers' childcare stress as well as the aims and research questions of the three studies conducted for the present thesis. I will then proceed to present the methods and results of the papers. Subsequently, toddlers' childcare stress will be assessed with an in-depth discussion of the observed cortisol movements and the child and childcare factors which could be linked to those movements. The thesis concludes with a discussion of ethical considerations, strengths, limitations, and further research needs.

1.2 Theoretical background

Toddlers' stress levels do not emerge in a vacuum but are connected to the environments they encounter, the caregiving they receive, their individual predispositions, and maturing processes (Engel & Gunnar, 2020). I will therefore firstly present research and theory on toddlers' development, their relationships, and settings, to prepare the backdrop for the subsequent introduction of the concept of stress.

1.2.1 Early child development in context

Toddlerhood is a period of rapid development. The foundation of the brain is constructed in the first few years of life in a constant interplay of the individual and the environment. This sets children up for a life-long health trajectory. Emotional and social development leads the way for the building of cognitive and language skills (Center on the Developing Child, 2007a). Important drivers of these processes are stable and warm relationships to adults who interact with children in an attuned, predictable, and reciprocal way (Center on the Developing Child, 2004b; La Paro & Gloeckler, 2015). Child development is a complex and interactive process (Hayes et al., 2017). This has been observed consistently by empirical research (Center on the Developing Child, 2004b) and is also well-theorized.

Bronfenbrenner's bioecological model of human development puts children's development in context and describes how different layers of linked systems interact with the individual (Bronfenbrenner, 1979; Hayes et al., 2017). The child is immediately located and participates actively in micro-systems (e.g., the family or the childcare center). These micro-systems are linked through meso-systems (e.g., parent-caregiver communication). Furthermore, exo-systems, contexts in immediate proximity to the micro-systems (e.g., parent workplace or educational politics), and superordinate macro-systems (social and cultural conditions in a society) impact children's lives, though in a more indirect manner as they shape the conditions for the micro- and meso-systems. Children's stress activity may differ according to the micro-system they are present in (Vermeer & Van Ijzendoorn, 2006) and since micro-systems are linked to meso-, exo-, and macro-systems, those may influence stress activity as well. One essential yet under-researched meso-system may be the initial transition between home and childcare. Starting in childcare may have a big impact on short- and long-term experience in childcare as well as children's coping abilities during future transitions (Brooker, 2008). Bronfenbrenner (1979) described how children's well-being in micro-systems is dependent on the quality of the meso-systems which link them and provided educational transition as an example. Bronfenbrenner called relationships between members of different micro-systems trans-contextual dyads. A larger number of trans-contextual dyads is called multiple links and is deemed advantageous. Child, parents, and caregivers form a three-person-system. The availability of a trusted parent, who is responding positively to the new context, during children's first encounters with the childcare center will likely give them a sense of security and thereby facilitate their exploration and the formation of relationships to new caregivers. The relationships in the three-person-system are ideally characterized by trust, openness, a balance of power,

positivity, reciprocity, goal-consensus, and an ongoing exchange of relevant information. Thus, the higher the quality and quantity of relationships in the meso-system, the more positive effects on children's well-being and the quality of caregiving can emerge. The transition is not a one-off event but a process that continues over time (Bronfenbrenner, 1979; Brooker, 2008; Hayes et al., 2017).

Bronfenbrenner recognized furthermore that systems are not static but change with time. He also stressed the importance of what he described as person (children's characteristics and resources) and process (nature, frequency and quality of activities and interactions between and among children and others). This extended bioecological model was labelled "process-person-context-time framework" and human interactions are its active ingredient (Bronfenbrenner, 1979; Hayes et al., 2017). Since environments and children's individual characteristics vary and constantly influence each other, developmental outcomes will vary to a great extent as well. Children's experience in a specific childcare context will differ based on their individual predisposition and learning history (Phillips et al., 2011). Among the factors that can affect children's experience and well-being in childcare are temperament, attachment security to parents, family socioeconomic status, age and age at childcare entry, gender and quantity of care (Bradley & Vandell, 2007) as well as the quality of relationships and caregiving (Gunnar & Donzella, 2002).

Early brain development is an interactive process between genetic predisposition, experience, and environment. Genes provide the information for the basic architectural plan of the brain, yet experience and environmental conditions have an immense impact on the emerging shape of brain architecture, for better and for worse (Center on the Developing Child, 2007b). Interactions with others are the active ingredient in this process and young children learn and

regulate their emotions most effectively with the support of caregivers (Center on the Developing Child, 2004b; Schore & Schore, 2008). Reciprocal, stable, and loving relationships in secure and stimulating environments promote healthy brain development. Frequent, chronic, or strong adversity in the absence of such relationships disrupts healthy brain development, as learning is overridden by wear-and-tear processes and the need to adjust to environments which are inherently unsupportive or dangerous. Brain growth accelerates rapidly from birth and countless neurons and synapses emerge. Those are subsequently pruned and formed into specialized brain circuits throughout infancy and toddlerhood. Exposure to or lack of certain experience, caregiving, nutrients, or toxins determine the strength and shape of brain circuits (Center on the Developing Child, 2004a, 2007b). Mental capacities develop in a hierarchical manner and go through phases of heightened sensitivity to environmental influence. For example, sensory processing and emotion regulation form the basis for higher-order cognitive functions (Gogtay et al., 2004). Hence, children have a need for enriching sensory and emotional cues way before they have use for academic stimulation. Weakness in lower-order brain circuits compromises the formation of higher-order brain circuits. For example, children who cannot regulate their emotions well will have greater difficulties to overcome social and cognitive challenges. Brain growth slows down considerably as children move into later stages of childhood and the brain circuits built in the early years form a life-long predisposition of mental and physical health. The quality of environments and caregiving children are exposed to during their first years of life have therefore profound impact on individuals' health and well-being during their entire lifespan. The brain stays malleable through life, but changing an existing neural foundation takes greater effort and longer time compared to forming a stable neural foundation from the beginning (Center on the Developing Child, 2004a, 2007b). Early

experience and environment also influence gene expression and the functioning of other physical systems, such as the cardiovascular system, the metabolic system, the neuroendocrine system and the immune system (Center on the Developing Child, 2010, 2020). The stress response is thought to be the major link between the environment, experience, and the body. Small and well-regulated doses of stress will promote optimal functioning of physical systems in resting state and during challenge. Large and uncontrolled stress however sets the body in a state of constant alert which causes wear-and-tear effects and maladaptation (Center on the Developing Child, 2020; Engel & Gunnar, 2020). Hence, stress plays a role in how children adapt and learn, and in turn their stage of maturation and will also influence their stress activity (Engel & Gunnar, 2020).

Some children may be more receptive to environmental influence than others (Belsky & Pluess, 2009; Center on the Developing Child, 2004a, 2014; Ellis & Boyce, 2011). Hence, some children may profit especially in high quality care but may also be influenced more negatively in low quality settings (Belsky & Pluess, 2009). Temperament could be an important indicator of this susceptibility. Temperament is a largely genetic trait and children range from being shy, passive, and emotionally reactive to being extrovert, active and contented. Children with a reactive and fearsome temperament have earlier been viewed to be more vulnerable to detrimental impact from stress-inducing environments. Researchers have recognized that those children may also be more likely to profit from highly supportive and enriching environments. Vice-versa, children with easier temperamental traits may be less receptive to environmental influence, which protects them to a certain extent from damage in adverse environments, but also limits their potential to accelerate under highly supportive conditions. This has been termed *differential susceptibility* or *biological sensitivity to context* (Belsky & Pluess, 2009; Ellis &

Boyce, 2011). The extent to which children's stress response is reactive versus regulated may also be a marker of susceptibility. Differential susceptibility is theorized to play a central role in an overriding evolutionary mechanism which ensures that a variety of phenotypes emerges and thereby increases the chances of species' survival in ever changing environments (Belsky & Pluess, 2009; Center on the Developing Child, 2004a, 2014; Ellis & Boyce, 2011). Children's needs are likely to differ according to their temperament and children with a more reactive and emotionally negative temperament may require greater sensitivity and considerations from caregivers (Center on the Developing Child, 2014).

I have now discussed how early child development is a complex and on-going process interwoven with environmental conditions and driven by interactions with others. In the further sections, I will explore the role of stress in early child development in greater detail. Before that, the context of early childcare will be introduced.

1.2.2 Early childhood education and care

In the recent years, many countries have recognized the importance of early childcare and increased their spending to provide childcare to a larger number of children and to increase the quality of provisions (OECD, 2017). On average, 46% of all two-year-olds were enrolled in early childcare in the OECD countries in 2018 (OECD, 2020). Yet, childcare quality and the number of hours children spend in childcare vary greatly and there is a great need to further promote the quality and equal accessibility of provisions (OECD, 2017). Norway is characterized by a high enrollment rate in full-time childcare (Statistics Norway, 2022e). Childcare is mostly organized into center care and there is also some family day care for young children. Childcare providers are both private and public and subsidized by the Norwegian state. Parental fees are comparably low and families with low income pay reduced fees (Engel et al., 2015). All childcare providers

adhere to the National Framework Plan for Kindergartens (Norwegian Directorate for Education and Training, 2017). A recent monitoring has found childcare in Norway to be of middle range quality (Bjørnestad & Os, 2018), and the quality and content of provisions has been observed to vary greatly (Lekhal et al., 2013).

Childcare quality is often described in terms of structural, process, and outcome quality. Structural quality concerns organizational factors like group size, teacher education, or number of children per caregiver. Certain structural elements of childcare are regulated in Norway. In toddler childcare, it is required to have one caregiver for every three children, and one pedagogue holding a bachelor's degree for every seven children (Jebsen & Norge, 2020). Structural quality creates the foundation for process quality (NICHD - Early Child Care Research Network, 2002). Process quality describes the characteristics of the relationships, interactions, and atmosphere in childcare (Pianta et al., 2016). Process quality is dependent on structural conditions, from the group-level and all the way up to the political level (Dalli et al., 2011). Process quality is not easily defined as it contains many aspects and varies with culture (Cadima et al., 2020). Frequently observed key characteristics in settings with high process quality are sensitive and stimulating interactions and warm, attuned relationships between caregivers and children, a wholesome understanding and consistent overlap of care and education and a strong collaboration between the home and the childcare center (Alvestad et al., 2019; Cadima et al., 2020; Dalli et al., 2011). Process quality is the active ingredient of childcare, i.e., how childcare affects children's well-being and development (Bronfenbrenner, 1979; Hayes et al., 2017; Pianta et al., 2016) and must be sufficiently good for children being able to benefit from childcare provisions (NICHD - Early Child Care Research Network, 2002). High process quality may be especially important during children's initial transition from home

to childcare (Brooker, 2008; Hayes et al., 2017). There is not enough systematic research on children's transitions to childcare. The initial separation from parents is likely to be challenging for them (Ahnert et al., 2004; Bernard et al., 2015). Qualitative studies suggest that having a key person among the staff and being accompanied by parents for a sufficient amount of time helps children to adjust to childcare (Brooker, 2008; Ebbeck & Yim, 2009; Markström & Simonsson, 2017; Undheim & Drugli, 2012). The third dimension of childcare quality is concerned with the developmental outcomes produced by childcare attendance. Early childcare has repeatedly been shown to have beneficial effects on children's well-being, learning and development (Camilli et al., 2010; OECD, 2017). If the structural and process quality of provisions are adequate, early childcare has the potential to promote cognitive (Burger, 2010; Camilli et al., 2010; NICHD - Early Child Care Research Network, 2002; Sammons, 2010), and social development (Bradley & Vandell, 2007; Broekhuizen et al., 2018). These beneficial effects are stronger with rising quality and children from risk backgrounds seem to profit more (Cadima et al., 2020). It is also important to note that extensive research could not document systematic adverse effects of early childcare attendance (Cadima et al., 2020; Lekhal, 2012; NICHD Early Child Care Research Network, 2005). Investing in early childhood, to promote the quality of relationships to parents and other caregivers and to reduce the prevalence of adversity has a great potential to deliver returns, both in the form of children's immediate well-being and their future health and functioning in society (Center on the Developing Child, 2007a, 2017; Knudsen et al., 2006). Providing high quality childcare can be an important part of this investment (Dalli et al., 2011). High quality childcare can support children's healthy brain development, while on the other hand, low quality childcare may be a risk factor for them (Bradley & Vandell, 2007; La Paro & Gloeckler, 2015). Investigating childcare stress may help us to understand children's experience

in childcare better and thereby to adjust it better to their needs (Vermeer & Van Ijzendoorn, 2006).

1.2.3 Stress in early childhood

Stress is a major pathway of how environmental cues get "under the skin" (Engel & Gunnar, 2020). Stress reactivity may also play a major part in children's inherent susceptibility to environmental influence. First, a clarification of the concept of stress and a simplified overview on its anatomy will be provided. Based on this discussion, I will elaborate how measuring stress levels may be a way of assessing children's experience in childcare. Then, previous research and knowledge gaps on toddlers' stress in childcare will be introduced.

What is stress?

Hans Selye is often mentioned as the founder of stress research, as he was one of the first scientists to recognize that psychosocial hardship can cause disease and malfunction in humans (Everly & Lating, 2019). "Stress" refers to a multitude of concepts in colloquial language. In the present thesis, the term stress is used to refer to the physical response to a stressor. A *stressor* is a biogenic (e.g. caffeine) or psychosocial (e.g. social encounter) stimulus that triggers stress (Everly & Lating, 2019). Psychosocial stressors are based on an individual appraisal of threat level and available coping resources (Gunnar & Herrera, 2013). A physical *stress response* is set off to put the body in a state where it can better cope with the stressor (Everly & Lating, 2019). The stress response can have lasting effects on the body and may alter target system or organs. This is referred to as *stress effects* (Everly & Lating, 2019). Stress effects range from learning and adaptation to damage. Short-term and modest stress responses help us to adapt to our environment and handle life's challenges (Godoy et al., 2018). Problems may arise when stress is too strong, chronic or impacts us early in development (Godoy et al., 2018).

The stress system

The human stress system is an extremely complex entity which involves many parts of the body and a large array of neural and hormonal communicating between those. The following explanations are therefore highly simplified.

Everly and Lating (2019) provide a six-step model of the human stress response. Firstly, a stimulus, of either biogenic or psychosocial nature, occurs. From here on, I am going to focus exclusively on psychosocial stressors. Secondly, the individual appraises the stimuli cognitively in the prefrontal cortex region of the brain together with a lot of other data, such as biological and psychological predispositions and available coping resources. The stimuli that become stressors can therefore vary greatly from individual to individual. Thirdly, if the individual perceives that the stimuli is threatening, overly challenging or otherwise undesirable, emotional arousal sets in. Fourth, neurological triggering mechanisms are set off in a complex interplay of different brain regions which lead to the fifth step, the actual stress response. The stress response can take three main pathways: The neural axes, the neuroendocrine axis, and the endocrine axes. Simplified, the stress response is a neural or hormonal signal or combination of both (Everly & Lating, 2019). Stress literature often portraits the sympathetic-adrenal-medullary system (SAM, the neuroendocrine axis) and the hypothalamic-pituitary-adrenal axis (HPA, one of the endocrine axes) as two main players of the stress response (Godoy et al., 2018). In simple terms, SAM is responsible for enabling us for an immediate and brief reaction to a stressor, such as fight or flight (Godoy et al., 2018; Gunnar & Herrera, 2013). HPA, which produces cortisol, delivers a slower and more sustained activation which helps us to stay vigilant and handle prolonged demanding situations (Godoy et al., 2018; Gunnar & Herrera, 2013; Suhonen et al., 2018). The HPA axis will be introduced in greater detail in the following section. Sixth, the stress response

activates target organs to set the body in a state where it is better able to cope with the stressor (e.g., increased blood flow and glucose levels) (Everly & Lating, 2019). Some stress activation is necessary for individuals to respond adequately to challenging situations and for learning processes (Suhonen et al., 2018). However, if the stress response is too intense or chronic, the targeted physiology may take damage. Unfavorable stress effects may include hypertension or inhibition of the immune system. In addition, stress can become self-perpetuating under chronic conditions, which is likely to further increase the risk for damaging effects (Everly & Lating, 2019).

The HPA axis

The HPA axis is a major player in the stress system and its end-product, cortisol, can be reliably measured in saliva (Kirschbaum & Hellhammer, 1994). The HPA axis is part of humans' regular metabolism and releases cortisol in a diurnal cycle, with the highest levels in the morning shortly after waking up and the lowest levels around midnight. Cortisol helps amongst other to steer our wake- and sleep-cycles. A balanced diurnal output of cortisol with a steep decline throughout the day has been linked to favorable physical and mental health outcomes (Gunnar & Herrera, 2013; Lindfors & Lundberg, 2002).

As part of the stress system, the HPA axis releases additional cortisol when triggered by a stressor. Increases of cortisol are recognized as an indicator for an individual's perceived psychological stress (Kirschbaum et al., 1990; Vermeer & Van Ijzendoorn, 2006). Hormone release of the HPA axis is a chain reaction with a negative feedback loop. When a stressor is perceived, the paraventricular nuclei (PVN) region of the hypothalamus releases two hormones, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which travel to the anterior pituitary gland, located just below the hypothalamus. There they cause the release of

adrenocortical trophic hormone (ACTH) which travels through the blood stream to the adrenal cortex, located on top of the kidney, and stimulates the production and release of cortisol. Cortisol molecules enter cells where they cause or inhibit the transcription of genes which leads to a mobilization in a variety of biological functions, such as the metabolism or the immune system. It takes about twenty minutes from HPA axis activation to full production of cortisol and even longer before cortisol takes full effect on cells. In the brain, cortisol binds to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). MR are being occupied under the baseline ebb and flow of cortisol and mediate restorative functions. GR begin to be occupied by cortisol molecules under additional cortisol release triggered by stressors. Activation of GR receptors aids the body to cope with stressors. In a balanced stress response, the release of cortisol inhibits the release of further cortisol and mediates the activity of other stress systems (Engel & Gunnar, 2020; Koss & Gunnar, 2018).

Calibration and dysregulation of the HPA axis

The HPA axis is thought to be a major link between environmental cues and child development. The HPA axis calibrates its own functioning and other physical functions according to the stressors which frequently occur in the individuals' environment (Engel & Gunnar, 2020). It is widely agreed upon that our stress system plays a major role in adapting us to our environment, yet it is unclear how this works exactly. Elaborate evolutionary frameworks model how the interplay between individual predisposition, environmental input and stress physiology leads to individuals' adaptation, yet they remain largely theoretical (Del Giudice et al., 2011; Ellis & Boyce, 2008; Koss & Gunnar, 2018).

Both diurnal functioning and stress reactivity of the HPA axis may become dysregulated under chronic stress and cortisol levels can become chronically high (hypercortisolism) or

chronically low (hypocortisolism) (Engel & Gunnar, 2020). It is unclear which circumstances lead to either hyper- or hypocortisolism. Hypocortisolism has been observed in the context of extreme stress (e.g., neglectful orphanage rearing). Hypo- as well as hyperreactivity of the HPA axis have been linked to unfavorable health outcomes. A balanced diurnal cortisol output as well as a moderate and subsequently sinking output of cortisol during stress exposure on the other hand seem to be crucial for health and optimal functioning (Engel & Gunnar, 2020).

Maturing of the HPA axis

Functioning of the HPA axis matures through the first years of life. Newborns' cortisol levels peak twice, approximately twelve hours apart, regardless of time of day. Even mild stressors such as a bath can produce elevations of cortisol in newborns. At three months of age, the characteristic morning peak and evening nadir start to emerge and the HPA axis becomes less reactive to stressors (Engel & Gunnar, 2020). The typical diurnal pattern has been observed in toddlers aged about twelve months (Watamura et al., 2004). HPA axis activity proceeds to mature and baseline cortisol levels continue to fall throughout toddlerhood (Blair et al., 2011). Afternoon naps result in a short and minor increase of cortisol levels (Mesas et al., 2022) and the diurnal ebb and flow of cortisol levels becomes more adult-like when children stop napping (Engel & Gunnar, 2020). Warm and stable caregiving is a potent buffer of the HPA axis' stress reactivity in early childhood. The presence of attachment figures also seems to contribute to a balanced diurnal output of cortisol levels (Gunnar & Donzella, 2002).

Types and effects of childhood stress

The Center on the Developing Child (2017) at Harvard University describes three categories of children's stress: Positive, tolerable, and toxic stress. Positive stress is described as

a mild and short activation of the stress system, which is necessary for healthy development. Childcare attendance likely has the potential to provide toddlers with challenges that elicit positive stress. Tolerable stress is a more serious or extended stress activation yet supporting relationships buffer for damaging effects. Secure social relationships have been observed to be a powerful down-regulator of HPA axis activity (Engel & Gunnar, 2020; Gunnar & Donzella, 2002), which means that children's stress in childcare may vary with their relationships to caregivers. Toxic stress is a chronic and severe stress activation in the absence of buffering relationships. This type of stress is severely disrupting children's development. Stress exposure works in an accumulative fashion. The likelihood of damages rises with frequency, duration, and severity of stress exposure and diminishing social support (Center on the Developing Child, 2017). Moderate and prolonged stress activation has been linked to disadvantageous effects on children such as inhibition of cognitive development (Phillips et al., 2011), internalizing problems (Saridjan et al., 2014), more frequent illness (Watamura et al., 2010), and greater vulnerability for future stress (Loman & Gunnar, 2010). While stress in childcare is unlikely to be toxic stress, we cannot rule out the possibility that stress could last for an extended time or occur in situations without proper social support.

Slight deviations from the diurnal rhythm of cortisol output have been observed in toddlers in childcare, meaning that they are on average slightly stressed in the childcare context. Some stress is likely beneficial, but the required amount may be quite low and deviations from the diurnal rhythm have been found to be unfavorable (Baumeister et al., 2014; Engel & Gunnar, 2020). We do not know enough about stress in childcare and cannot merely assume that the cortisol elevations observed in childcare are positive stress. It is unclear what the effects of long-term, moderate childcare stress are (Vermeer & Van Ijzendoorn, 2006). Smaller elevations and

adherence to the diurnal rhythm are preferable (De Vet et al., 2023). Hence, childcare practice adjusted to children's need should produce smaller cortisol elevations and less deviation from the diurnal rhythm.

Assessing toddlers' cortisol levels in childcare

Measuring toddlers' cortisol levels can be a way of assessing their experience in childcare (Kirschbaum et al., 1990; Vermeer & Van Ijzendoorn, 2006) and may thereby help us to better understand and improve childcare practice. Toddlers, especially the youngest, cannot express yet how they feel about childcare. Furthermore, observed behaviors do not always reflect children's distress reliably (Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006) and physiological measures may therefore be better markers of stress (Vermeer & Van Ijzendoorn, 2006). Assessment of cortisol in saliva is a reliable measure of the unbound "active" cortisol levels in the blood stream (Kirschbaum & Hellhammer, 1994). These levels follow a diurnal, sinking pattern but have been repeatedly shown to rise in response to psychosocial stimuli (Kirschbaum & Hellhammer, 1994). Deviations from the circadian rhythm of cortisol levels are considered a biological marker for stress (Vermeer & Groeneveld, 2017). Previous investigation of stress reactivity through animal models, psychological experiments, and stress measurement in naturalistic contexts has shown that situations which are characterized by social threat, novelty, uncontrollability, and unpredictability are most reliable to produce elevations in cortisol levels (Kirschbaum & Hellhammer, 1994; Vermeer & Van Ijzendoorn, 2006). For instance, the Trier Social Stress Test is a stress-inducing protocol which reliably causes cortisol levels to rise to twice to four times their baseline level in adults (Kirschbaum et al., 1993). The test is comprised of a wait period and public speaking or calculation task under critical audience acclaim and produces significant and reproduceable cortisol elevations. Kirschbaum et al. (1993)

hypothesized that the social threat provoked by high ego involvement and anticipation of negative consequences during the task was the main cause for the distinct stress reaction in subjects.

There is a high inter- and intraindividual variability in both baseline cortisol activity and stress reactivity. The circadian rhythm varies between individuals (Kirschbaum & Hellhammer, 1994; Vermeer & Van Ijzendoorn, 2006). Also, cortisol output due to a stress response varies across individual and situations. Kirschbaum et al. (1990) describe how cortisol levels controlled for time are a function of state (external stimuli), trait (individual predisposition), and state x trait. Cortisol levels have been observed to show higher intraindividual consistency in the morning, but later in the day they vary more due to external stimuli and the interaction of individual predisposition and external stimuli. Under especially demanding challenges, variations in cortisol levels may be more closely related to external situations than to individual characteristics (Kirschbaum et al., 1990). To account for high intra- and interindividual variability, repeated measurements of cortisol levels are required on several according time points on more than one day. Repeated measurements also allow for a combination of cross-sectional and longitudinal investigation of cortisol levels (Kirschbaum & Hellhammer, 1994). When evaluating cortisol levels in different contexts, baseline levels need to be measured first. For instance, measures of home cortisol levels applied as baseline enables us to make intraindividual comparisons between the childcare and home context (Vermeer & Van Ijzendoorn, 2006).

HPA axis activity is interconnected with children's predispositions (trait) and characteristics of the childcare environment (state) (Kirschbaum et al., 1990; Vermeer & Groeneveld, 2017). By measuring cortisol levels in childcare and at home, we may be able to

identify groups of children, who are especially prone to stress in childcare and childcare practice which increases or decreases stress in toddlers. Including a high number of participants may increase our chances of finding meaningful relations between cortisol levels, and child, family, and childcare factors.

1.3 Previous research on toddlers' stress in childcare

To find relevant research studies, I applied a broad search strategy on Google Scholar and the Scopus and PubMed databases, using the search terms "cortisol" and "childcare" as well as "cortisol" and "toddler" in combination. In addition, I used a snowball-strategy and looked for relevant citations in highly relevant papers and also looked at subsequent citations of those papers. A final literature search was performed in May 2023. As of today, there are only a handful of research studies measuring toddlers' cortisol levels in childcare and few research studies that measure toddlers' cortisol levels longitudinally during the initial transition to childcare. Furthermore, two review papers, one meta-analysis and one paper that combines a review and a meta-analysis on children's cortisol levels in childcare could be found. All studies have been conducted in Europe and North America. Drugli et al. (2018) have conducted the only Norwegian study on the topic. It is not entirely clear how cortisol levels move between morning and afternoon in childcare compared to at home. Cortisol levels during transition to childcare are not sufficiently investigated, as there are only very few transition studies with toddlers as participants. There is no longitudinal research on toddlers' cortisol levels in childcare. Except for transition studies, all previously conducted studies are cross-sectional and only few of them include an in-depth investigation of home cortisol levels. It is furthermore unclear how cortisol levels in childcare and at home are linked to child, family, and childcare factors.

The majority of studies has found elevated cortisol levels in toddlers in childcare compared to home (Drugli et al., 2018; Groeneveld, et al., 2010; Ouellet-Morin et al., 2010; Sumner et al., 2010; Tervahartiala et al., 2020; Tervahartiala, Kortesluoma, et al., 2021; Tervahartiala, Nolvi, et al., 2021; Vermeer et al., 2010; S. Watamura et al., 2003). However, there are also studies which have not found a difference between cortisol levels at home and in childcare (Suhonen et al., 2018; Vermeer et al., 2010). Tervahartiala et al. (2020) observed higher cortisol levels as well as a slight elevation of afternoon cortisol levels in a control group of stay-at-home toddlers. Only a couple of studies so far have measured toddlers' cortisol levels in the evening at home after a day in childcare. These studies found that cortisol levels to dropped below their initial morning level once children returned home (Groeneveld et al., 2010; Sumner et al., 2010; Tervahartiala et al., 2020; Tervahartiala, Kortesluoma, et al., 2021; Tervahartiala, Nolvi, et al., 2021). Two studies have included a follow-up measurement when children were three years old. Ouellet-Morin et al. (2010) found sinking cortisol levels between morning and afternoon in both childcare and at home at age 3, while Tervahartiala, Kortesluoma et al. (2021) observed cortisol levels to continue being elevated in childcare. It is not clear yet how toddlers' cortisol levels vary with context and time, and we need to investigate further.

All reviews and meta-analyses conclude that there is a general tendency for children to have elevated cortisol levels in childcare (De Vet et al., 2023; Geoffroy et al., 2006; Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006) and the toddler age group may be especially prone to show these elevations (Geoffroy et al., 2006; Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006).

In addition, I have found four studies measuring toddlers' cortisol levels during their initial transition to childcare. Ahnert et al. concluded that toddlers were especially activated

during the first two to three weeks separated from parents, but less so when parents were present with them in the childcare center and after a couple of weeks and months of attendance (Ahnert et al., 2022; Ahnert et al., 2004). Drugli et al. (2023) described a similar curve-linear pattern of stress activation with low stress levels on the initial days together with parents in childcare, high levels in the first weeks separated from parents, and comparably lower stress levels after three months in childcare. Bernard et al. (2015) observed increasingly higher cortisol elevations during children's first ten weeks in a new childcare setting. In this study, childcare-aged children (18-60 m) were more prone to show cortisol elevations than infants and school-aged children.

Researchers have also studied the relation between cortisol levels and secondary measures. It is recognized that toddlers' cortisol levels in childcare are linked to child and family factors as well as childcare factors, but research findings are contradictory (Vermeer & Groeneveld, 2017). The use of different measurement tools and metrics for child and childcare factors and different procedures of measuring and analyzing cortisol levels may be partly responsible for diverging findings. This also aggravates the comparison of studies. Furthermore, a considerable number of previous studies included fewer than a hundred participants, which may have decreased their chance of detecting relations between cortisol levels and other factors.

Gender has not been connected to cortisol levels so far (Drugli et al., 2018; Groeneveld et al., 2010; Sumner et al., 2010; Vermeer et al., 2010). When it comes to age, Watamura et al. (2010) found that the tendency of showing afternoon elevations of cortisol in childcare peaks at approximately two years of age and subsequently lowers. Other studies did not observe a link between toddler age and cortisol levels (Groeneveld et al., 2010; Legendre, 2003; Sumner et al., 2010; Vermeer & Van Ijzendoorn, 2006). Investigations of child temperament have produced mixed findings. Tervahartiala et al. observed children with higher levels of surgency to have

higher cortisol levels both in childcare and at home at two years of age, but surgency was not related to cortisol levels at three years of age. Furthermore, they found no connection between cortisol levels and negative affectivity (Tervahartiala, Kortesluoma, et al., 2021; Tervahartiala, Nolvi, et al., 2021). Watamura et al. (2010) reported that children with higher social fearfulness and less peer play had overall higher cortisol levels and steeper afternoon inclines of cortisol levels in childcare. Ouellet-Morin et al. (2010) found a steeper increase of cortisol levels for children with more internalizing behavior at two years of age (but not at three years of age), but no connection between cortisol levels and externalizing behavior and effortful control. On the other hand, Drugli et al. (2018) did not find a connection between cortisol levels and infant adaptability and Vermeer et al. (2010) could not observe a link between cortisol levels and an overall score of children's temperamental difficultness. When it comes to cognitive functioning, no relations have been found between cortisol levels and language abilities and cognitive skills so far (Suhonen et al., 2018). Results are unclear when it comes to family factors. Drugli et al. (2018) reported that cortisol levels and maternal postnatal depression and maternal education level were unrelated and also Sumner et al. (2010) did not find family income to be relevant for cortisol levels. Suhonen (2018) found also no connection between cortisol levels, family income, parental education, and number of children in the family. However, none of those studies focused on a sample with low socioeconomic status. Toddlers from a background with high socioeconomic risk may show a different stress activity in childcare (Berry et al., 2014). Children's stress in childcare is likely to vary with quality of care and researchers have therefore investigated various aspects of childcare quality, with ambiguous results: Vermeer et al. (2010) found sinking cortisol levels in centers with an above average global quality score (ECERS-R) and mildly rising levels in centers with a score below average. Groeneveld et al. (2010) linked

higher total cortisol production to lower caregiver sensitivity in home-based childcare. They found rising cortisol levels in center-based care with lower global quality scores (ECERS-R, IT-CC-HOME). Drugli et al. (2018) and Sumner et al. (2010) could not establish a link between children's cortisol levels and process quality, measured with CLASS Toddler and ITERS-R respectively. When it comes to structural quality of childcare, Legendre (2003) found higher cortisol levels in large groups, in groups with great age differences, when only a small play area per child was available and when an unexpected high number of adults was present. Legendre reported no relations between cortisol levels and boy-girl-ratio, caregiver professional experience, age range between caregivers, staff turn-over and caregivers' familiarity with the group. There are further non-findings for group size (Drugli et al., 2018; Tervahartiala et al., 2020; Vermeer et al., 2010) and number of children per caregiver (Drugli et al., 2018; Vermeer et al., 2010). Only few studies have investigated the relation between cortisol levels and quantity of care. Drugli et al. (2018) found rising cortisol levels for children who spent eight or more daily hours in childcare while children who spent less time in childcare showed no increase in cortisol levels. Tervahartiala et. al (2020) found, after performing a robust regression analysis, that children who attended childcare on more days in a month had higher cortisol levels. Previous research on the link between cortisol levels and child, family and childcare factors has produced ambiguous results. Hence, the matter needs to be investigated further, preferably with a high number of participants and in a both longitudinal and cross-sectional manner.

1.4 Aims of the thesis

Overall aim of the thesis

The present thesis examined toddlers' cortisol levels through novel or little researched perspectives, namely during the initial transition from home to childcare, longitudinally through a whole year in childcare and cross-sectionally with a thorough investigation of home cortisol levels in a comparably large sample of children. The first objective was thereby to observe if and when stress occurred, if childcare stress spilled over into the home context, and if there were tendencies of stress accumulation or habituation throughout the year in childcare. Furthermore, the present thesis tried to contribute to entangle the link between cortisol levels and a variety of childcare, child, and family factors. We sought to identify childcare practice that induced stress in toddlers and groups of children who were more stressed in childcare than others in order to adjust childcare better to toddlers' needs. The overall research question was: How do toddlers' cortisol levels change cross-sectionally and longitudinally during transition, in childcare and at home and how are these changes related to child, family, and childcare factors?

Aim of Paper 1

We examined the different stages of the initial transition between home and childcare and investigated if the experience of transition varied with basic child and childcare factors.

(1) How do cortisol levels change during the day (morning, afternoon, and evening) and during the different phases of transition (together with parents, separated from parents, and after the first month in childcare)?

(2) How are gender, age at childcare entry, number of siblings, and group size related to cortisol levels during transition?

Aim of Paper 2

We observed children's cortisol levels cross-sectionally from morning to afternoon in childcare and at home. We compared cortisol movements in the two contexts and investigated if child, family, and childcare factors were related to a certain pattern of cortisol movement in childcare, at home or in both contexts.

(1) How do cortisol levels change between morning and afternoon in childcare and at home in a comparably large sample of 320 children?

(2) Do the patterns of cortisol level change between morning and afternoon in childcare and at home depend on child factors (age, gender, temperament, well-being), family factors (maternal education), childcare quality (number of children per caregiver, number of pedagogues among the staff, classroom organization, interaction quality), or childcare quantity (number of daily hours spent in childcare)?

Aim of Paper 3

We investigated toddlers' cortisol levels longitudinally throughout a year in childcare and examined how child, family and childcare factors were related to both daily and longitudinal movements cortisol movements.

(1) How does toddlers' daily pattern (morning, afternoon, evening) of cortisol activity in childcare look like?

(2) How does cortisol activity change during a year in childcare?

(3) How are childcare quality (group size, number of pedagogues among the staff, process quality), child factors (temperament, well-being), and family factors (maternal education) related to toddlers' daily cortisol activity and changes in cortisol activity during the year in childcare?

2 Methods

2.1 Design

We used quantitative methodology and a longitudinal and cross-sectional design to answer our research questions. Study 1 and 3 are longitudinal investigations where we follow our participants throughout their initial transition from home to childcare and through a whole year in childcare respectively. Study 2 is a cross-sectional comparison of cortisol levels in childcare and at home.

2.2 Participants

The present thesis and its three studies are part of Thrive by Three, a large research study and quality enhancement program for toddler classrooms in South-Eastern and Central Norway (Lekhal et al., 2020). Researchers, municipalities, and childcare employees cooperated to strengthen process quality in toddler classrooms and to study the effect of childcare quality on children's development and well-being. Thrive by Three had a cluster-randomized trial design with half of the childcare centers receiving an extensive quality-building program throughout a whole year. The other half of the centers comprised a wait-list control group.

Seven municipalities in Norway were chosen to participate in Thrive by Three. Childcare centers with toddler groups in these municipalities could opt into Thrive by Three. Seventy-eight centers decided to do so, and 794 caregivers gave their informed consent to fill out questionnaires about themselves and the participating children. Caregivers informed parents of Thrive by Three and parents were able to give informed consent to their child's participation in the study. When parents shared custody, both had to consent to their child's participation in the study. Thrive by Three, including the cortisol studies which are part of the present thesis, was

approved by the Regional Committee for Medical and Health Research Ethics (REK) and the Norwegian Center for Research Data (NSD).

Participants in the studies of the present thesis were chosen in the following manner: We randomly chose four to five of the centers enrolled in Thrive by Three from each municipality. Those centers are described as "cortisol centers" from here on.

Study 1

For the first study, all children with valid consent who started childcare in one of the cortisol centers in fall 2018 were eligible. Three cortisol centers did not have eligible children and were therefore excluded from study 1. We randomly chose up to nine children from each remaining cortisol center. This resulted in the participation of 135 children. After a drop-out of fourteen children, we received saliva samples of 121 children. Thereof, two children were excluded because of a pattern of exceedingly high cortisol values. Study 1 included 119 participants. Since the Thrive by Three intervention had not started yet, we included children from both control and intervention group centers in study 1.

Study 2 and 3

For study 2 and 3, we randomly chose up to thirteen children with valid consent from each of the cortisol centers. In study 2, both children from intervention group and control group centers were eligible. Preliminary statistical analysis (linear mixed model with individual as random effect, log10-transformed cortisol level as the dependent variable and control vs. intervention group as a two-category covariate controlled for age and gender) revealed that there was no difference in cortisol levels between the groups. This resulted in the enrollment of 344 participants. After a drop-out of five children, we received saliva samples from 325 children. We excluded a further five children, because of a pattern of improbably high cortisol values. Study 2

included 320 participants. Study 3 had a longitudinal design with several cortisol measurements throughout a year and we could not readily exclude interference of the parallel ongoing Thrive by 3 quality intervention with cortisol levels. We therefore chose to only include children from control group centers in study 3. This resulted in a sample of 159 children. After a drop-out of two children, we received samples of 157 children. Thereof, we excluded one child because of a pattern of exceedingly high cortisol values. Study 3 included 156 participants.

Detailed demographic data on the participants can be found in the respective papers. Participating families had an above average annually income and parental education (Statistics Norway, 2022a, 2022d). Single parent households and minority language backgrounds were under-represented (Statistics Norway, 2022b, 2022c). Hence, our sample is characterized by a low socioeconomic risk and representativeness may be limited to middle class populations.

2.3 Cortisol measuring

Sampling schedule

The HPA axis activity shows great inter- and intraindividual variations and it is therefore important to measure cortisol repeatedly and across several days, preferably with a high number of individuals (Engel & Gunnar, 2020; Hanrahan et al., 2006; Kirschbaum & Hellhammer, 1994). Hence, we scheduled saliva sampling on two sequential days, when possible, to minimize the effect of intraindividual differences, and included a higher number of participants than comparable studies. We did not sample saliva in the evenings in the middle of the year (T2). At this time point, families had to take four home samples on the weekend, and we concluded that it would be an unreasonably high participant burden for children and parents to take an additional four evening samples. Table 1 displays the sampling schedule for all three studies. Because of an

already high participant burden, we were not able to control for sleep times and food and medicine intake.

Table 1

Time schedule of saliva sampling

August 2018 (T0) Study 1				September 2018 (T1) Study 1: Follow-up (F) / Study 3		January 2019 (T2) Study 2 /Study 3				June 2019 (T3) Study 3	
Acclimatization: Day 2 (A1) and 3 (A2) in childcare with parents		Separation: Day 1 (S1) and 2 (S2) in childcare without parents									
10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00*	10.00*	10.00	10.00
15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00*	15.00*	15.00	15.00
18.00*	18.00*	18.00*	18.00*	18.00*	18.00*					18.00*	18.00*

* Home measurement

Study 1 measured cortisol levels of children starting childcare in fall 2018, when parents were still present in childcare (acclimatization) and on the first days without parents in childcare (separation). Most of those children started childcare in August, at least four weeks before saliva sampling in September, which was applied as a follow-up measurement in the context of study 1. When children started later than August in childcare, their measurements from the acclimatization and separation phase were included in study 1, but they did not participate in study 2 and 3. Study 2 is a cross-sectional study of cortisol levels in childcare and at home in January 2019. Study 3 is a longitudinal study which followed children through a year in childcare and included the measurement in September 2018 and January and June 2019.

Sampling procedure

Before each measurement, childcare centers received two sampling kits for each child, one for collecting saliva samples in childcare and one for collecting saliva samples at home. Caregivers distributed home kits to parents. The kits contained swabs to collect saliva, empty test

tubes, and instructions on when and how to sample saliva. The author of the thesis was available by phone in case caregivers and parents had questions about the sampling procedure. We used the Salimetrics' SalivaBio Children's Swab, a tool designed for saliva sampling from young children and validated for cortisol measurement (Salimetrics, 2019). Test tubes were marked with a color indicating the time of day, a number indicating the day, and an additional identification number indicating the participant and time-point of sampling. This identification number was only recognizable for the author of the thesis. Caregivers took saliva samples in a playful manner and placed the swabs in the test tube corresponding to the sampling time point. Parents took samples at home accordingly and delivered them to their childcare center the following day. We did not use stimulant. Children were not pressured to participate in the sampling procedure. Childcare centers froze all samples in household freezers. Thrive by 3 staff picked up the samples from the childcare centers in a timely manner. Samples were stored shortly in locked household freezers at the university sights in Trondheim and Oslo and immediately sorted, cataloged, and sent to the cortisol laboratory at the University of Trier, Germany.

Analysis

The laboratory analyzed the saliva for cortisol using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA) (Dressendörfer et al., 1992). The laboratory ensured quality of test results amongst other by analyzing every sample twice and calculating a mean as well as a coefficient of variation (CV) between the measurements. Depending on the cortisol value of the sample, the CV was allowed to range from 10-15%. Samples with cortisol values below 2 nmol/l were allowed to have a higher CV. Samples with improbably high or low cortisol values as well as a too high CV were

re-analyzed. 90 % of the cortisol values in our data set are a mean of two immunoassay results. Approximately 10 % of the samples we sent to the laboratory did not contain enough saliva for double immunoassay and were only analyzed once. However, this should not impact our data since random measurement error plays a minor role in the applied type of immunoassay (Kirschbaum & Hellhammer, 1994). Some test tubes did not contain enough saliva for analysis. Others did produce an implausibly high cortisol value or in very rare cases an extremely low cortisol value also after re-testing. This might have been due to either sickness or contamination or medication interfering with immunoassay (Hanrahan et al., 2006). After guidance by the St. Olav's hospital in Trondheim and the cortisol laboratory at the University of Trier, we decided to exclude all cortisol values below 0.001 nmol/l and above 30 nmol/l, as they likely reflected irregularities. We also excluded some samples with values in the normal range if individuals had a pattern of otherwise improbably high cortisol values. A detailed overview of the status of all samples can be found in the method sections of the three papers. All samples were destroyed after analysis.

2.4 Secondary measures

We included a wide range of child, family, and childcare factors to detect relations with cortisol levels. Reliability and validity of psychometric tools were discussed and found to be at least acceptable for each applied tool.

2.4.1 Child factors

Parents answered electronic questionnaires at T1 on children's age in months, gender, and number of siblings.

Temperament

Children's temperament was assessed with the Emotionality Activity Shyness (EAS) Temperament Survey. Mothers answered this questionnaire at T1. The EAS measures temperament on a 5-point Likert scale (1 = not typical, 5 = very typical) with 20 items (e.g., "this child is always on the go") loading onto four subscales (Emotionality, Shyness, Activity, Sociability). A higher sum score indicates a higher congruence to the trait in question. Mathiesen and Tambs (1999) explored the psychometry of the EAS in a study with more than 900 Norwegian toddlers and reported adequate stability over time, acceptable internal consistency, and applicability of the EAS for young children. For the present thesis, the Sociability scale showed low internal consistency in both study 2 and 3 and was therefore excluded. The remaining subscales had acceptable Cronbach's alpha values in study 2 and 3 respectively (Emotionality: 0.71, 0.80, Activity: 0.77, 0.77, Shyness: 0.71, 0.73).

Well-being in childcare

Caregivers who knew the child best answered the Leiden Inventory for the Child's Well-being in Daycare (LICW-D) (De Schipper et al., 2004) at T1 on a child-basis. The LICW-D consist of 12 items (e.g., "this child is actively seeking contact to other children") rated on a 6-point Likert-scale (1 = applies never, 6 = applies always) loading onto the factor "this child enjoys attending the childcare center". A higher mean score indicates a higher well-being in childcare. For the Norwegian translation, we adjusted the 6-point Likert-scale to a 5-point Likert scale because point 3 (applies regularly) and point 4 (applies often) were linguistically indistinguishable in Norwegian (Van Trijp et al., 2021). The Cronbach's alpha was 0.87 and 0.81 in study 2 and 3 respectively. Van Trijp et al. (2021) conducted a psychometric study on the

LICW-D and reported appropriate construct and concurrent validity and deemed the instrument fit for application with Norwegian toddlers.

2.4.2 Family factors

Maternal education

Mothers provided their level of education at T1 (0 = no university education, 1 = university education).

2.4.3 Childcare factors

Process quality

We assessed the quality of interactions between toddlers and caregivers with the Classroom Assessment Scoring System (CLASS) Toddler (La Paro et al., 2012). CLASS Toddler is an observational tool rating caregivers' behaviors in toddler classrooms on eight dimensions loading onto two domains: Emotional and Behavioral Support (EBS) and Engaged Support for Learning (ESL). The EBS domain consists of five dimensions: Positive Climate, Negative Climate, Teacher Sensitivity, Regard for Child Perspective and Behavioral Guidance. The ESL domain consist of three dimensions: Facilitation of Language and Development, Quality of Feedback, and Language Support. Observers had to take a two-day course and reach at least 80% inter-rater agreement while rating three master-coded videos to become certified. Booster sessions were arranged before each observation day. The certified observers observed the classroom for three loops of 15 minutes on a morning at T1 and T2. The interrater reliability Intraclass Correlation Coefficient of the EBS domain was 0.88 and 0.91 for the ESL domain in the Thrive by Three study. Each dimension was rated on 7-point Likert-scale. A score from 1-2 indicates low quality, a score from 3-5 indicates middle-range quality and a score from 6-7 indicates high quality. The score of the Negative Climate dimension was reversed, with 7

representing no incidence of negative climate. The dimension scores were then averaged across the three loops of observation and subsequently averaged into a domain score. CLASS Toddler has been reported to have criterion-based validity and applicability for European contexts (Cadima et al., 2022; Slot et al., 2017) and showed a high internal consistency when it was applied in study 3 (EBS: Cronbach's alpha = 0.89, ESL: Cronbach's alpha = 0.95) and study 2 (EBS: Cronbach's alpha = 0.90). We investigated only the EBS domain in study 3. We found this domain most suitable for investigation, as the HPA axis is highly sensitive to social interaction (Gunnar & Donzella, 2002).

We furthermore measured the occurrence of organization/disorganization in childcare groups with the Life in Early Childcare Programs Scale (LECP) (Kontos & Wachs, 2000; Wachs et al., 2004). Organization and disorganization describe the degree to which group units are calm, spatially, and temporally well-structured, and predictable versus unrestful and unpredictable. The LECP measures organization with 16 items (e.g., "we rarely have time to tidy up and clutter accumulates") on a 5-point Likert scale (1 = never applies, 5 = always applies) with higher scores representing higher levels of disorganization. Caregivers who knew the child best answered the questions on a child-basis at T1. The LECP showed good internal consistency when applied in study 3, with a Cronbach's alpha of 0.89. Wachs et al. (2004) found a high test-retest stability for the LECP as well as acceptable internal consistency.

Structural quality

Head teachers provided structural information on the childcare group units at T1. We assessed the number of children in the group (group size), the number of full-time caregivers, and the number of pedagogues holding at least a bachelor's degree among the caregivers. We calculated the ratios of pedagogues among the staff (number of pedagogues/ number of full-time

caregivers) and number of children per caregiver (number of children in the group/ number of full-time caregivers).

Childcare quantity

Parents answered electronic questionnaires at T1, providing the average number of hours children spent in childcare each day (0 = up to 8 hours a day, 1 = more than 8 hours a day).

2.5 Statistical analyses

The linear mixed model

We chose linear mixed models for all investigations of cortisol levels. Mixed models are able to handle dependencies between observation such as in nested data and repeated measures (Thoresen & Gjessing, 2012). Because of a high intraindividual variability of cortisol levels (Hanrahan et al., 2006) it is not advisable to impute missing cortisol data. Mixed models can use each data point, also when individuals have missing values (Krueger & Tian, 2004). Analyses were mainly performed in SPSS 26 and 27. The likelihood ratio tests in study 2 were performed in STATA 17.

Preliminary analyses

Distribution of raw cortisol data was positively skewed and therefore log10-transformed prior to analyses. Before including children of the Thrive by Three intervention group in study 2, we tested if group enrollment made a difference for cortisol levels by applying a linear mixed model with individual as random effect, log10-transformed cortisol level as the dependent variable and group membership as a two-category covariate while controlling for age and gender. The Thrive by Three intervention had not started at the time of saliva sampling for study 1. We could not find a difference between the groups in terms of their cortisol levels and included participants from the intervention group in study 2. Study 3 was conducted with children from

the control group only, because we were unsure if we could exclude effects of the intervention on cortisol level with certainty when it came to investigating cortisol data from a whole year.

Study 1

We applied a linear mixed model with cortisol level (nmol/l, log10-transformed) as the dependent variable, individual as random effect, and time of day (morning, afternoon, evening) as three-category covariate and day (acclimatization day 2 and day 3, separation day 1 and 2, follow-up) as a five-category covariate including the interaction between time of day and day. The two measurement days in the follow-up phase were coded as one day, because there was essentially no difference between them. Normality was checked by visual inspection of Q-Q plots. To study the relation of child, family, and childcare factors to cortisol movements, we entered those secondary measures in a dichotomized form as two-category covariates one at a time into the mixed model, including their two- and three-way interactions with time of day and day. We regarded two-sided p-values <0.05 to represent statistical significance and reported 95% confidence intervals.

Study 2

Cortisol (nmol/l, log10-transformed) was entered as the dependent variable into a linear mixed model with individual as a random effect and time of day (morning, afternoon) and context (childcare, home) as two-category covariates, as well as the interaction of time of day and context. The two measurement days in each context were coded as one day. We controlled for age and gender. Normality of residuals was checked visually with Q-Q plots. We found slight deviations from normality and performed the analysis therefore again with 2000 bootstrap replications (BCa). The analysis results did not change considerably under bootstrapping. Two investigate the relation between child, family, and childcare factors and movement of cortisol,

we entered those secondary measures one at a time as covariates with their two- and three-way interactions with time of day and context into the linear mixed model, while controlling for age and gender. Subsequently, these expanded models were compared to the initial model with likelihood ratio tests. In study 2, we regarded two-sided p-values <0.05 to be statistically significant and reported 95% confidence intervals where relevant.

Study 3

We applied a linear mixed model with cortisol (nmol/l, log10-transformed) as the dependent variable and individual as random effect. Time of day (morning, afternoon, evening) and month (September, January, June) were entered as three-category covariates, including their interaction. The two measurement days of each month were coded as one day, as there was no qualitative difference between them. In addition, we controlled for age and gender. Normality was checked by visual inspection of Q-Q plots. We found slight deviations from normality when controlling for age and repeated the analysis with 2000 bootstrapping repetitions and the bias corrected and accelerated method (BCa). Analysis results were essentially the same after bootstrapping. We also included a robustness check with home cortisol levels to ensure that the observed cortisol movements were indeed related to the childcare context. Cortisol measurements (nmol/l, log10-transformed) from two weekend days in January at home were entered as the dependent variable into a linear mixed model with individual as random effect and time of day (morning, afternoon) as a two-category covariate. The two weekend days were coded as one day. To investigate the relation of child, family, and childcare factors to cortisol movements in childcare, we included secondary measures one by one as covariates into the first linear mixed model, together with their two- and three-way interactions with time of day and month. In addition, we controlled for age. We regarded two-sided p-values <0.01 to represent

statistical significance in study 3, due to multiple hypotheses. Because of a lack of previous research, we could not form a hypothesis on how we should cortisol levels expect to change throughout the year in childcare and how these changes would be related to family factors and therefore chose to operate with a more conservative level of significance.

3 Results

The general research question we investigated was: How do toddlers' cortisol levels change cross-sectionally and longitudinally during transition, in childcare and at home and how are these changes related to child, family, and childcare factors?

This was studied in the following manner: In the first study, we investigated the changes in cortisol levels in 119 children starting childcare in August 2018. We focused on three different stages of transition: the acclimatization (parents were still accompanying children in childcare), the separation (the first days of children being without parents in childcare), and a follow-up phase (a month after childcare entry). Days were labelled in the following way: A1 = second day together with parents in childcare, A2 = third day together with parents in childcare, S1 = first day without parents in childcare, S2 = second day without parents in childcare, F = follow-up. The aim of the study was to see how cortisol levels varied throughout the transition and to identify stages which are especially challenging. In addition, we investigated how cortisol levels were related to certain child and childcare factors (age, gender, number of siblings, group size). Study 2 took a cross-sectional focus on the cortisol levels (morning, afternoon) of 320 children in childcare and at home in the middle of the year in childcare (January). We furthermore investigated how child (age, gender, temperament, well-being), family (maternal education), and childcare factors (process quality, group organization, number of children per caregiver, number of pedagogues among the staff), were related to cortisol levels both in childcare and at home. By studying the relation of these factors to cortisol levels both in childcare and at home, we intended to distinguish different baseline cortisol levels from greater vulnerability for stress in childcare and we wanted to see if children carried stress from childcare to the home context. In study 3, we investigated 156 children's cortisol levels (morning, afternoon, evening) longitudinally

throughout a year in childcare (September, January, June) to gain insight into how cortisol levels developed through a whole year and if children habituated to childcare or if they accumulated stress. Also, we investigated the relation between daily and longitudinal movements of cortisol and child (temperament, well-being), family (maternal education), and childcare factors (process quality, group size, number of pedagogues among the staff).

3.1 Cross-sectional change of cortisol levels

Cortisol levels varied significantly with the time of day on all points of measurement. During transition to childcare, cortisol levels were significantly elevated in the afternoon compared to morning on all days of measurement except for the days of the acclimatization phase. Afternoon elevations of cortisol levels peaked in the separation phase, with raw levels rising considerably from morning to afternoon (31.5 %, 42.5%) on the respective days. Afternoon cortisol levels were still significantly elevated compared to morning in the follow-up phase. Cortisol levels fell to a low level in the evening on each day of measurement. Estimated marginal means and p-values of change for cortisol levels in the transition study can be found in figure 4 and 5 in paper 1. Furthermore, cortisol levels differed significantly between the childcare and home context depending on time in the middle of the year in childcare. Cortisol levels rose significantly between morning and afternoon in childcare. Cortisol levels fell significantly between morning and afternoon at home. Morning cortisol levels did not differ between childcare and home. Afternoon cortisol levels were significantly higher in childcare compared to home. Estimated marginal means and p-values corresponding to change across the childcare and home context can be found in Figure 2 in paper 2. Cortisol levels varied with time of day throughout the whole year in childcare. Afternoon cortisol levels were slightly yet not significantly elevated compared to morning cortisol levels in the beginning and middle of the

year, but there was no difference in the end of the year. Note that in this study, the level of significance was set to 0.01. Cortisol levels declined steeply between afternoon and evening in the beginning and end of the year in childcare. Figure 3 in paper 3 shows the estimated marginal means and the according p-values of change.

Summarized, cortisol levels were elevated in the afternoon in childcare compared to morning on most measurement days and fell to a low level in the evening on all measurement days. At home, cortisol levels followed a typical diurnal decline between morning and afternoon. The highest afternoon elevations were found in the separation phase during the transition to childcare.

3.2 Longitudinal change of cortisol levels

Cortisol levels also showed changes across longer periods of time under transition and a year in childcare. Morning cortisol levels did not change throughout the transition. Afternoon cortisol levels were significantly increasing compared to A1 throughout the transition, with a peak on day S2. Evening cortisol levels were slightly but significantly decreasing throughout the transition. When investigating children's cortisol levels during a whole year in childcare, morning levels rose slightly, afternoon cortisol levels stagnated, while evening cortisol levels rose significantly between the beginning and the end of the year.

3.3 Relations of changes in cortisol levels to child, family, and childcare factors

Children's age, well-being, and activity level as well as the number of children per caregiver and the degree of organization in group units were found to be related to cortisol levels. Other child and childcare factors as well as family factors (maternal education) could not be linked to cortisol levels. To ease and visualize comparisons, all secondary measures were

divided at their median either before (paper 1) or after (paper 2 and 3) linear mixed model analysis.

Age

During transition to childcare, age produced a significant main effect ($p = 0.001$) and a significant interaction effect with time of day ($p = 0.001$). Younger children (aged up to 13 months) had higher overall cortisol levels. The difference was especially pronounced for the evening measurements and for the afternoon measurement of the follow-up phase. Magnitude and change of cortisol levels across the childcare and home context varied with child age ($p < 0.001$) in study 2. This p-value was produced by a likelihood ratio test. Younger children (up to 26 months) had overall higher cortisol levels in childcare. Morning cortisol levels at home did not differ according to age, yet younger children had higher afternoon cortisol levels at home. Hence, older children showed a steeper decline of cortisol levels at home. Findings regarding age are visualized in figure 6 and 7 in paper 1 and figure 3 in paper 2.

Well-being

Children's well-being in childcare ($p < 0.001$) was significantly related to the magnitude of cortisol levels and change of cortisol levels throughout the year in childcare. Children with lower well-being scores (cut-off at the median) had overall higher cortisol levels at all points of measurement. They also showed an incline of morning and afternoon cortisol levels, while children with a higher well-being score had stagnating morning cortisol levels and decreasing afternoon cortisol levels across the year. In addition, children with lower well-being showed a steeper increase of evening cortisol levels between the beginning and end of the year in childcare. Graphs of cortisol levels according to well-being can be found in figure 4 and 5 in paper 3.

Activity level

Children's activity level ($p = 0.009$) was significantly related to the magnitude of evening cortisol levels throughout the year in childcare. Children with a lower activity score (cut-off at the median) had overall higher evening cortisol levels. This result is visualized in figure 6 and 7 in paper 3.

Number of children per caregiver

The magnitude of children's cortisol levels in childcare and at home varied slightly with the number of children per caregiver in their group unit ($p = 0.031$). This p-value was produced by a likelihood ratio test. Children who attended a group with more than three children per caregiver had slightly higher overall cortisol levels in childcare, and slightly lower cortisol levels at home. Cortisol levels according to number of children per caregiver can be found in figure 4 in paper 2.

Group organization

The magnitude of cortisol levels in childcare and the magnitude and change of cortisol levels at home could be linked to the organization score of group units ($p = 0.012$). This p-value was produced by a likelihood ratio test. Children who attended a group with a higher group disorganization score (above 1.94 on a scale up to 5) had distinctly higher cortisol levels in childcare. They had also slightly higher afternoon cortisol levels at home. Hence, children in group units with a lower disorganization score showed a steeper decline of cortisol levels at home. There was no difference for the magnitude of morning cortisol levels at home. Cortisol levels according to group organization are visualized in figure 5 in paper 2.

4 Discussion

The present thesis investigated the full-day cortisol levels of toddlers in childcare and at home during transition to childcare and throughout a whole year in childcare. In addition, we studied the relation between cortisol levels, child, family, and childcare factors. The goal was to observe when stress occurred, if childcare stress was carried over into the home context and if there were longitudinal tendencies of stress accumulation or habituation. A further objective was to identify groups of children who are more likely to experience stress in childcare, as well as to pinpoint childcare practices that induce stress. The overall research question was: How do toddlers' cortisol levels change cross-sectionally and longitudinally during transition, in childcare and at home and how are these changes related to child, family, and childcare factors?

4.1 Summary of findings

Cross-sectional change of cortisol levels

We consistently found elevated or plateaued afternoon cortisol levels compared to morning cortisol levels. This indicates that children were on average stressed in the afternoons in childcare. Rising cortisol levels in childcare were contrasted by sinking cortisol levels at home, which points toward that the observed afternoon stress activations are indeed induced by childcare. Children seemed to experience the childcare and the home context differently. Consistently low evening cortisol levels even after distinct afternoon cortisol elevations suggest that children relaxed once they came home, and that stress activation was limited to the childcare context. Overall, daily patterns of cortisol levels followed a diurnal decline, despite afternoon cortisol elevations, which indicates that the stress activation in childcare was not chronic. Age was significantly linked to stress levels. We observed higher overall cortisol levels for younger children both in childcare and at home. This may be a matter of maturation of the HPA and also

hint towards a greater immaturity of skills necessary to navigate the childcare context and a less rapid recovery from childcare stress in the younger age group (Watamura et al., 2004). During transition, the youngest children had distinctly higher afternoon cortisol levels after the first month of childcare attendance, which may indicate that they needed longer time to settle in at childcare. The height of stress levels in the middle of the year varied with certain factors of childcare quality. We observed higher cortisol levels in childcare for children in groups with more than three children per caregiver and a lower degree of organization. This indicates that a lower availability of adult support and lack of stability and calmness may have been stressful for children. Said stress activation was most pronounced in childcare but may have spilled over into the home context to some degree at this point in time.

Longitudinal change of cortisol levels

We also observed change in cortisol levels over time. Our starting point of investigation was the initial transition from home to childcare and we followed our children throughout a whole year (beginning, middle, end) in childcare. Morning cortisol levels rose slightly during the transition and throughout the year, which may hint towards a somewhat earlier onset of stress activation with time. The closing gap between morning and afternoon cortisol levels in the end of the year is caused by an increase in morning cortisol levels. Afternoon cortisol levels rose distinctly during the transition with a peak on the second day separated from parents and then stagnated throughout the year. Children seemed to be more relaxed when parents were present in the childcare center, but the first days separated from parents were clearly stressful. The drop in afternoon cortisol levels after a month indicates that most children had settled in at childcare at this point in time. However, afternoon cortisol levels were elevated or plateaued compared to morning cortisol levels on all measurement occasions, which points towards an enduring stress

activation in the afternoons in childcare. Evening cortisol levels declined during the transition but rose between the beginning and end of the year. Children may have been increasingly more able to bounce back from stress activation during the transition phase. However, as the year proceeded, childcare stress seemed to accumulate and spill over into the home context. Longitudinal patterns of cortisol level change varied with certain child factors. Children showed a different profile of longitudinal stress activity according to their well-being reported by caregivers. Children with high well-being showed signs of habituation to childcare with stagnating morning cortisol levels, decreasing afternoon cortisol levels and overall lower and less steeply increasing evening cortisol levels. At the same time, children with lower well-being seemed to be more and increasingly stressed throughout the year with distinctly rising morning, afternoon, and evening cortisol levels. This results in a clear gap by well-being at the end of the year, where children with lower well-being showed distinctly higher stress levels throughout the day. In addition, one aspect of temperament was found to be related to cortisol level change. Children with lower activity levels may have been less able to bounce back from childcare stress at home as indicated by higher evening cortisol levels.

Those findings will be addressed in greater detail in the following sections. Firstly, I will discuss whether the measurement of cortisol levels in saliva is a reliable and valid indicator of perceived stress. Secondly, children's stress across context and time will be reflected upon in more depth and linked to a broader scientific discourse on toddlerhood and early childcare attendance. The discussion chapter concludes with a review of ethical considerations, strengths and limitations of our studies, and future research needs.

4.2 Reliability and validity of cortisol levels as an indicator of perceived stress

Measuring cortisol levels in saliva is a reliable method of assessing the level of unbound and thereby active cortisol molecules in the blood stream. Random measurement error has been observed to play a minor role in this process (Kirschbaum & Hellhammer, 1994). However, compliance to the sampling procedure and adequate quality of sampling tools and laboratory analysis need to be ensured (Hanrahan et al., 2006; Kirschbaum et al., 1990). We instructed caregivers and parents in detail on the sampling procedure, applied a swab which was validated for the sampling of saliva and subsequent analysis for cortisol (Salimetrics, 2019), and selected a laboratory with solid experience in the field of cortisol measurement. However, deviations from the sampling schedule as well as a lack of data on sleep and mealtimes, and medication intake may have influenced the reliability of the cortisol measurements. These issues will be discussed in the limitations section.

Cortisol levels may be valid indicator of an individual's perceived psychological stress if certain things are kept in mind. Cortisol levels (both in baseline and during a stress response) have a high intraindividual and interindividual variability (Engel & Gunnar, 2020). Therefore, we looked at cortisol levels in an intraindividual and interindividual average (estimated marginal mean derived from linear mixed models) (Kirschbaum & Hellhammer, 1994). We were not only looking at the absolute average cortisol level, but at change in average cortisol levels across several time-points, both cross-sectional throughout a day and longitudinal at corresponding time points across several days. It must be clarified that cortisol elevations are not only caused by perceived psychological stress, but also by other triggers such as physical activity, infections and as a reaction to waking up (Engel & Gunnar, 2020). Some diseases and traumatic experiences may also inhibit cortisol elevations in stressful situation (Gunnar & Vazquez, 2001). Looking at

cortisol levels in individuals isolated from other information is therefore not a valid indication of perceived psychological stress. To ensure to gain an understanding of individuals' perception of how stressful a context is, we measured cortisol in many individuals which are subjected to the same state, namely childcare. We then looked for subgroups according to state and trait in these average measurements across time. I can argue in favor of cortisol levels being a valid marker of perceived psychological stress when investigated in this manner by looking at different types of validity. Cortisol elevations have been consistently observed in the presence of potential psychological stressors, especially threats to the social self, novelty, unpredictability and uncontrollability in animal studies, laboratory experiments and naturalistic settings (Dickerson & Kemeny, 2004; Kirschbaum & Hellhammer, 1994; Vermeer & Van Ijzendoorn, 2006). Hence, cortisol levels are widely applied and recognized as an indicator of perceived psychological stress by researchers (Kirschbaum et al., 1990; Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006) and possess therefore face validity. I can critically discuss construct validity. For instance, the Trier Social Stress Test has shown construct validity with cortisol elevations being linked to self-reported psychological and physiological anxiety symptoms during the experiment in adults and adolescents (Vors et al., 2018; Wu et al., 2019). However, children's emotional expressions and their cortisol output may not be consistently linked (Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006). Measuring cortisol levels in childcare as a marker of perceived stress may possess discriminant validity, as toddlers have been observed to show elevated cortisol levels in the childcare context, which is theorized to have a higher prevalence of potential psychological stressors (Tervahartiala et al., 2020), compared to the home context. Cortisol levels as an indicator of perceived psychological stress may also possess predictive validity. High cortisol levels over time can be linked to negative health outcomes

(Engel & Gunnar, 2020). However, it is unclear if psychological strain is causing those health outcomes or if other additional factors are involved and there is insufficient research on the long-term effects of toddlers' cortisol elevations in childcare. To conclude, cortisol levels in the individual are not a valid indicator for perceived psychological stress, however cortisol levels across an average of many individuals who face a similar condition may be a valid indicator for it.

4.3 Childcare stress across context and time

4.3.1 Childcare stress across context

Diverging patterns of cortisol level change in childcare and at home indicate that children experienced these contexts differently. We found evidence of stress activation in childcare, especially in the afternoons, and relaxation at home on all points of measurement. After conducting the three additional studies connected to this thesis, it can be stated that the majority of studies on toddlers' cortisol levels in childcare detected signs of a stress activation in childcare (Drugli et al., 2018; Groeneveld et al., 2010; Legendre, 2003; Ouellet-Morin et al., 2010; Sumner et al., 2010; Tervahartiala et al., 2020; Watamura et al., 2003), also in the European context (De Vet et al., 2023). Our findings indicate that the childcare environment is more demanding for toddlers than the home environment (Vermeer & Van Ijzendoorn, 2006) and aspects of low quality amplified the contrast between the settings.

Bronfenbrenner's process-person-context-time framework helps to pin-point the differences between the childcare and the home environment. The home and the childcare center are two different micro-systems and part of the context children grow up in. There is a shift in process between these systems: In the childcare center, children are separated from parents and instead part of a peer group and cared for by several childcare employees. Most children spend

more than eight hours daily in childcare in Norway (Statistics Norway, 2022e). Young children rely on adult support to make sense of experiences and to regulate their feelings (Center on the Developing Child, 2004b; Schore & Schore, 2008). However, access to and quality of adult support is likely to vary between the childcare center and the home and caregiving in childcare may not be equally effective in regulating children's stress (Vermeer & Groeneveld, 2017). The separation from parents may be stress-inducing in itself for toddlers (Gunnar & Herrera, 2013). In addition, parents' absence means that children are lacking an important instance of stress regulation (Gunnar & Donzella, 2002).

Childcare typically has a lower caregiver availability compared to home. The link between higher cortisol levels and a higher number of children per caregiver indicates that lower caregiver availability was stressful for our participants. The adult-child-ratio is an element of structural quality and regulated to a maximum of three children per caregiver for the toddler age group in Norway (Ministry of Education and Research, 2020). In previous research, a higher number of children per caregiver has been linked to lower occurrence of comfort, support, well-being, and behavior guidance both on an individual and group level (De Schipper et al., 2006; Løkken et al., 2018), and less opportunities to engage in positive peer interactions (Iluz et al., 2016). However, research findings regarding structural quality are not straightforward and a high adult-child-ratio does not guarantee high interactional quality (Pianta et al., 2016; Slot et al., 2015). We found that already small increases in the adult-child-ratio were linked to higher stress levels in children. Even though we cannot determine an active ingredient, this observation shows that caregiver availability was likely important for our toddlers. In daily practice, the number of children per caregiver may at times be a considerably higher than three, especially in the mornings and afternoons, because of long opening hours, lunch breaks, organizational tasks, or

sick leave (Os & Hernes, 2019; Oslo kommune, 2022). Our findings underline that the mandated adult-child-ratio of three children must not be exceeded and that it needs to be provided during all opening hours of childcare centers.

Furthermore, also dyadic caregiving likely varies between home and childcare. Caregivers may not be able to comfort and support children equally adequately as parents, especially during toddlers' initial time childcare (Elfer et al., 2012). There are many observations of warm and attuned relationships between toddlers and caregivers, however caregivers have been found to be tuned-in less well to young toddlers with emerging social and verbal skills compared to older children and there may be tendencies of overlooking toddlers' silent cues (Bratterud et al., 2012; Gevers Deynoot-Schaub & Riksen-Walraven, 2008). Especially routine care situations, which offer unique opportunities for sensitive and comforting one-on-one interactions, may often be low in interactional quality (Bratterud et al., 2012; Cadima et al., 2022; Klette et al., 2018). Interactions with adults are closely related to children's stress regulation (Gunnar & Donzella, 2002) and we intended to measure the quality of said interactions with CLASS Toddler. To our surprise, we have not found any link between CLASS Toddler scores and children's cortisol levels. CLASS Toddler is a group measure and averages the quality of all observed interactions. However, process quality is likely to vary across individual children and activities in the same classroom (Cadima et al., 2022; Pianta et al., 2003; Slot & Bleses, 2018). Hence, group-averaged scores may only in part reflect the process quality individual children actually receive. Social regulation of stress may occur between individuals (Gunnar & Donzella, 2002). It is therefore possible that CLASS as a group measure did not adequately capture the interactions which were relevant for stress regulation (Guerrero-Rosada et al., 2021; Vermeer & Groeneveld, 2017). There may have also been too little variation in quality

in our sample to make a difference for stress levels (Vermeer & Groeneveld, 2017). Some researchers argue that tools for measuring process quality should be more nuanced and conducted on an individual level for greater validity (Burchinal, 2018; Weiland et al., 2023).

At the same time, our findings show that elements of group-level caregiving may also be important for children's stress regulation. Nine to twelve children seem to be the most common group size for toddlers in Norwegian childcare centers (Utdanningsdirektoratet, 2021), as opposed to the home context where the average child in Norway has few siblings (Statistics Norway, 2022b). Nine to twelve children are considered to be a large group for toddlers, and considerably larger group sizes have been observed in a recent quality monitoring (Bjørnestad & Os, 2018). Caregivers need to not only be sensitive towards the individual child, but they also need to keep group dynamics and routines in mind (La Paro et al., 2012; Pianta et al., 2008). Greater teacher sensitivity towards the group has been observed to be related to higher levels of collaborative peer play (Van Schaik et al., 2018) and greater likelihood of secure attachment to caregivers (Ahnert et al., 2006). Well-flowing routines are likely facilitating positive caregiver interactions and may provide children with a sense of security (Abrahamsen, 2015; Hagström, 2010; Klette et al., 2018). We found distinctly higher stress levels for children in a group characterized by a lower degree of organizational flow. Elements of disorganization are noise, crowding, traffic and lack of spatial and temporal stability and predictability (Wachs et al., 2004). The relation between group organization and stress levels is discussed in depth in paper 2. Even though I cannot determine an active ingredient, the sheer extent of difference in childcare stress levels between children in well-organized and mildly disorganized group units shows that organizational flow was highly important to our toddlers. This finding supports the notion that group-level interactions are an essential part of process quality (Van Schaik et al., 2018).

Interactions with peers have been frequently suspected to illicit stress in toddlers (Engel & Gunnar, 2020; Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006). Social and communicational skills are still emerging during toddlerhood, which is why peer contact may be demanding to navigate, and conflict may easily arise (Gevers Deynoot-Schaub & Riksen-Walraven, 2006). Peer interactions can trigger complex learning processes, which may involve stress activations (Suhonen et al., 2018). Interacting and playing with peers is essential for the development of social, communicational, and cognitive skills (Alvestad, 2010; Engdahl, 2011; Singer et al., 2012; Van Schaik et al., 2018). Children tend to experience joy from playing with other children (Drugli, 2017) and positive peer interactions may even down-regulate stress levels (Engel & Gunnar, 2020). Caregivers need to support toddlers in their contact with peers (Gloeckler & Cassell, 2012; Singer et al., 2012), both proactively to facilitate play and avoid conflict (e.g., by splitting the group into smaller units, clear rules and consistent routines) and reactively (e.g., by helping children to negotiate and to take peers' perspective) (Gloeckler & Cassell, 2012; La Paro et al., 2012). Previous studies have observed inadequate quality when it comes to supporting peer relations (Os & Hernes, 2019).

Continuing with Bronfenbrenner's process-person-context-time framework, toddlers as persons move through maturity levels (time) in which the childcare center may be a more challenging micro-system than experienced by other age groups. As discussed earlier, children's social, emotional, and cognitive skills unfold during toddlerhood, all while already navigating complex relationships in a group setting with peers and caregivers and in the absence of parents (Gunnar & Donzella, 2002; Shaffer & Kipp, 2014). Children's baseline cortisol levels have been observed to decrease during toddlerhood (Blair et al., 2011; Simons et al., 2015; Watamura et al., 2004). Our findings are in line with this as we have observed lower overall cortisol levels for

older toddlers during transition to childcare and in the middle of the year in childcare, as well as a steeper decline of cortisol levels across the day at home. One reason for the repeatedly observed decrease in baseline cortisol levels may be the emerging of capacities which help to better regulate emotions and navigate stressful situations, such as effortful control (Simons et al., 2015). However, this cannot be the only explanation for lowering set-points of cortisol levels, as it would mean that younger toddlers are consistently more stressed compared to older toddlers, which seems unlikely. Profound maturing processes of the brain and endocrine systems are likely at play (Watamura et al., 2004). While cortisol levels were lower for the older toddlers in our studies, they still showed a stress activation in childcare which indicates that childcare continued to be a challenging context. Previous research points towards that afternoon elevations of cortisol levels in childcare are pronounced in toddlerhood and that they may begin to subside in the course of the preschool years (De Vet et al., 2023; Geoffroy et al., 2006; Ouellet-Morin et al., 2010; Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006). To summarize, while there are likely complex developmental processes at play when it comes to the lowering of baseline cortisol levels in the early years (Watamura et al., 2004), children‘s afternoon cortisol elevation in childcare may become flatter as they develop capacities to better navigate the childcare context (De Vet et al., 2023; Ouellet-Morin et al., 2010; Tervahartiala, Kortesluoma, et al., 2021). Some children may continue to be stressed throughout preschool and into the school years (De Vet et al., 2023; Geoffroy et al., 2006).

We found that toddlers experienced the childcare and home context differently and the childcare environment is likely more challenging for them (Vermeer & Van Ijzendoorn, 2006). At the same time, childcare attendance holds many potential benefits for young children, such as new relationships and vast opportunities to play and learn (Brooker, 2008). Our findings indicate

that childcare should be better adjusted to toddlers' unique needs and point directly toward the importance of a sufficient caregiver availability and well-flowing group routines, which ensure calmness, stability, and predictability. Hence, group-level processes were likely important for children's stress regulation. Previous research has demonstrated clearly that dyadic interactions with adults play a central role in children's stress regulation (Gunnar & Donzella, 2002) but we may have not been able to capture the quality of those interactions with CLASS Toddler. Caregivers need to be aware of toddlers' maturity level and recognize subtle means of communication (Bratterud et al., 2012; Gevers Deynoot-Schaub & Riksen-Walraven, 2008), provide closeness and warm interactions, also in routine care situations (Cadima et al., 2022; Gevers Deynoot-Schaub & Riksen-Walraven, 2008), and support children to navigate peer contact (Gloeckler & Cassell, 2012). All of this requires highly attentive and empathic caregivers and preferably they should have specialized education on childcare for the toddler age group (Os & Hernes, 2019). The stress activation we observed in our studies was most pronounced in the afternoons. Caregivers should therefore focus on calm activities and warm interactions in the afternoon, especially for children who expect to stay many hours (Drugli et al., 2018). Childcare leadership needs to ensure the presence of sufficient staff until closing time (Os & Hernes, 2019). Furthermore, shorter hours in childcare, if feasible for parents, may help to keep stress levels in check (Vermeer & Van Ijzendoorn, 2006).

4.3.2 Childcare stress across time

We started our longitudinal investigations of stress during one-year-olds' initial transition from home to childcare and subsequently followed them and their two-year-old peers throughout a whole year in childcare. The transitional stress pattern had a curve-linear shape. From there on, children proceeded to develop different stress profiles according to their well-being. I will first

discuss the stress pattern we observed during the transition phase and then proceed to address the longitudinal stress pattern in the following section.

Stress during transition to childcare

The stress pattern we observed during transition suggests a few key points. Firstly, the initial days with parents present in childcare likely represented an important time window for the establishment of relationships. Secondly, the first days of separation from parents were tiresome, probably for most children. Finally, it appears that most children had settled down after a month in childcare. However, there also seemed to be children who continued to struggle. Our findings are in line with the results of Ahnert et al. (Ahnert et al., 2022; Ahnert et al., 2004) and Drugli et al. (2023), who reported similar curve-linear pattern of stress activation during transition. Our study largely replicated the results of previous studies but also delivered new insights by detecting a high afternoon cortisol level for children below 14 months after the first month in childcare. Younger children may need more time to settle in at childcare. Transitioning is likely an extended process with significance for future transitions and short-and long-term well-being in childcare (Brooker, 2008). In the recent years, childcare researchers have argued how priming activities before starting childcare, such as weekly visitation hours at the center or a home visit from caregivers, could ease children's transition to childcare (Fabian & Dunlop, 2007; O'Connor, 2013). Such activities may not yet be common in Norway (Drugli et al., 2017). Many of the participating centers may have offered three days of acclimatization with parents, which has been conventional practice in Norway due to work leave regulations (Drugli et al., 2017). The stress pattern observed during transition to childcare suggests that there could be benefits when increasing the number of acclimatization days (Ahnert et al., 2022; Ahnert et al., 2004; Drugli et al., 2023). The quality of the transition likely consists of both structural and processual aspects.

Structural factors, such as the number of days parents are present, or the provision of priming activities, can be regulated. For instance, the municipality of Trondheim has recently introduced a new transition model called “Little and new in childcare” which entails weekly visitations before attendance starts as well as a five-day parent-active acclimatization phase (Drugli et al., 2023; Løkås, 2023). Caregivers and parents in Trondheim have consistently reported positive experiences with this model (Drugli, 2020). While it is indeed important to provide children with enough time to become familiar with childcare, the quality of contact between children, caregivers and parents matters as well. Both parents and caregivers need to make an active effort to establish relationships (Degotardi & Pearson, 2009; Ebbeck & Yim, 2009). A key-person approach may facilitate a higher process quality during the transition (Elfer et al., 2012). The first days without parents seemed to be highly stressful and children require may a lot of support from caregivers on these days. Short days are preferable in this phase (Drugli et al., 2023). Our findings indicate that most but possibly not all children seemed to be less stressed after the first month in childcare. Caregivers need to keep in mind that transition is likely an extended process and provide calm activities in the afternoons in the first half of the childcare year (Degotardi & Pearson, 2009; Undheim & Drugli, 2012). Drugli et al. (2023) collected qualitative data from caregivers and parents parallel to measuring children’s cortisol levels in their recent transition study. Caregivers reported that they did not always understand children’s signals at first but gradually learned to decipher their communicative cues during the first month in childcare. They also described that children did not express much tiredness in childcare. However, parents consistently stated that children were very tired after childcare. Hence, caregivers may need to attend more closely to children than children themselves may signal and also communicate well with parents in order to learn to interpret children’s cues better. Younger children and children

who are passive and withdrawn are likely to require more support in their transition (Datler et al., 2010).

Stress during a year in childcare

On average, there seemed to be some stress accumulation as the year in childcare proceeded and stress may have spilled to a greater degree into the home context in the end of the year. This notion is underlined by a further finding: children showed obverse profiles of the longitudinal stress pattern according to their well-being in childcare as reported by caregivers. Children with very high well-being had decreasing stress levels and seemed to habituate to childcare during the year, while children with lower well-being became increasingly stressed and showed signs of a stress accumulation. It has to be noted that the median of the well-being score was high (4.33 of 5) and we therefore compared children with an upper medium well-being score to children with a very high well-being score. Caregivers may have systematically overrated children's well-being, or we may have failed to include children with low well-being into our studies. However, it is noteworthy that we see such a clear difference in the longitudinal stress patterns for well-being groups at such a high cut-off point and I wonder how children with low well-being are faring in childcare. Lowering afternoon cortisol levels throughout the year indicate that children in the very high well-being group became increasingly more at ease with and less stressed in childcare. They also showed some increase in evening cortisol, but to a lower extent. We may have observed a habituation process (Engel & Gunnar, 2020). Rising cortisol levels across the year including less ability to bounce back from afternoon cortisol elevations in the evening at home may reflect an upregulation of cortisol levels over time in the medium well-being group because of stress accumulation. This is also termed allostatic load (Blair et al., 2011). In addition, children with a lower activity level also seemed to have a greater tendency to

carry stress from the childcare context to the home context. Childcare attendance may be stressful in itself (De Vet et al., 2023) and it is not clear if toddlers fully habituate to childcare (Engel & Gunnar, 2020). However, our finding implies that there are differences in how children adjust to the childcare context over time, and that there are groups who seem to habituate or accumulate stress to a greater degree.

The link between increasingly higher stress levels and lower well-being supports the argument that cortisol levels are a valid marker of perceived psychological stress. Conclusions regarding causality cannot be made. Nevertheless, few would disagree that well-being has a high value in itself. I will take a closer look at well-being as it was the factor that separated children into different longitudinal stress profiles. Well-being in toddler childcare is not yet clearly defined and not sufficiently researched (Van Trijp, 2023). We measured well-being with the LICW-D which assesses caregivers' general impression of the degree to which children feel at ease with caregivers, peers, and physical surroundings across different everyday situations (De Schipper et al., 2004; Van Trijp, 2023). Recognizing and supporting groups of children who are vulnerable to low well-being as well as understanding how to promote well-being may help to lower stress levels in childcare over time. Firstly, well-being is likely connected to children's characteristics. High shyness, high emotionality and low sociability have been linked to lower well-being in toddler childcare (De Schipper et al., 2003; De Schipper et al., 2004; Van Trijp et al., 2021). Furthermore, young children with behavioral problems have been observed to have lower well-being in childcare (De Schipper et al., 2004; Gevers Deynoot-Schaub & Riksen-Walraven, 2006). Secondly, childcare practice that promotes well-being needs to be identified. Well-being has been observed to be closely tied to the quality of children's relationships with peers and caregivers (Fukkink, 2022; Seland et al., 2015). In addition, well-being may vary by

situation, with higher levels during exploration, play and positive social interaction and lower levels during routine situations and transitions (Fukkink, 2022; Seland et al., 2015). Toddlers' well-being in childcare is not yet well-studied and a variation in definitions, measurement and observation tools in previous research makes it difficult to draw distinct conclusions on how to promote well-being (Fukkink, 2022; Van Trijp, 2023). High well-being in childcare may arise from a "goodness of fit" between the individual characteristics of children and childcare contexts (Van Trijp, 2023) which further aggravates its investigation. There is a clear need for more research on well-being and the link between stress and well-being in childcare.

Well-being, stress, temperament, and childcare quality are likely interconnected, and our findings cannot entangle this function either. The two different longitudinal profiles according to well-being may show different paths of adjusting to childcare. Those who experience lower well-being in childcare have often been observed to be children with difficult temperament and behavioral problems (Van Trijp, 2023). It is possible that other disadvantageous factors such as an insecure attachment style or a failed transition, also contribute to lower well-being (Brooker, 2008). My concern is that there is a gap in how much stress children experience in childcare. On opposite ends of the average stress pattern, there may be children who over time experience positive stress and mastery in their adjustment to childcare, while other children may continue to struggle and be exposed to considerably higher levels of stress. Process quality may vary with child-caregiver dyads in childcare (Slot & Bleses, 2018). Children may illicit different kinds of caregiving from childcare employees according to their characteristics (De Schipper et al., 2004, Pianta et al., 2003). Children with a balanced and sociable temperament, more confidence and better language skills may be easier and pleasant to interact with and receive more positive attention from caregivers. They may also be more able to approach caregivers to have their needs

met. Children with internalizing or externalizing behavior tendencies and difficult temperaments on the other hand may be at higher risk for being overlooked or receive negative feedback more frequently. This may be a factor that contributes to diverging profiles of longitudinal stress. More longitudinal research on toddlers' stress in childcare is necessary. Future studies must identify characteristics of children who become increasingly stressed as time goes by and what helps or hinders their adjustment to childcare. Meanwhile, existing knowledge delivers a good basis for the claim that caregivers need to reflect on how they care for young, passive, quiet, shy, little sociable, fearful, and highly emotional children (Datler et al. 2010). They need to include them in positive peer interactions, exploration, and play (Seland et al., 2015). Caregivers must detect peer conflict and aimless wandering and ensure that children spend as little time as possible in these states (La Paro et al., 2012). In addition, quality in routine situations needs to be improved (Klette et al., 2018; Os & Hernes, 2019).

4.3.3 What type of stress did we observe?

It is recognized that some stress is necessary for healthy human development. To be beneficial, stress activations need to be time-limited, low, and ideally experienced in the presence of supportive relationships (Center on the Developing Child, 2017; Suhonen et al., 2018). Childcare attendance holds many unique opportunities for children to play, learn and form social bonds (Brooker, 2008). It is therefore reasonable to assume that positive stress occurs in childcare. However, only a minimal amount of stress may be required for optimal development (Engel & Gunnar, 2020). Research regarding the relations between cortisol levels in childcare and child, family and childcare factors has produced ambiguous results, however the relations that have been found point away from childcare stress being merely positive. Higher stress levels have been connected to less peer play (Watamura et al., 2003), crowding (Legendre, 2003),

lower caregiver sensitivity (Groeneveld et al., 2010) and lower global quality (Groeneveld et al., 2010; Vermeer et al., 2010). The relation between higher cortisol levels and lower well-being and higher disorganization observed in our studies also diverts from the notion that childcare stress is solely positive. Research has repeatedly connected lower and sinking diurnal cortisol output to higher levels of well-being and health (Engel & Gunnar, 2020; Lindfors & Lundberg, 2002) Our and other studies have frequently observed elevated levels of afternoon cortisol. The repeatedly observed stress elevations likely constitute time-limited, moderate stress on average and we conclude that cortisol levels in childcare should preferably be low and follow a sinking pattern (De Vet et al., 2023). Even though the observed stress was not chronic, some stress accumulation seemed to occur. It is not clear which long-term effects cortisol elevations in childcare have (Vermeer & Van Ijzendoorn, 2006) and the present thesis cannot answer this question either. Moderate stress in children has been connected to unfavorable outcomes (Loman & Gunnar, 2010; Phillips et al., 2011; Watamura et al., 2010). At the same time, early childcare attendance does not seem to have systematic negative effects on children's development (Cadima et al., 2020). Future research needs to investigate the effects of toddlers' stress in childcare on developmental outcomes over time.

4.4 Ethical considerations, strengths, and limitations

Ethical considerations

Thrive by Three, including the cortisol studies were introduced to and approved by the NSD and the REK. Participation in the Thrive by Three study was informed and voluntary for parents and caregivers. When parents shared custody, both of them had to agree to participation in order to enroll their child into the study. Withdrawal and selective participation were possible at any stage of the study without having to name reasons. Caregivers and parents were instructed

to refrain from saliva sampling if children were uncomfortable with the procedure. Children who repeatedly expressed discomfort during saliva sampling were removed from the cortisol study (n =1). In compliance with guidance from the NSD, we stored the test tubes only shortly in a locked freezer before sending them to the laboratory. Anonymity and confidentiality were ensured. The identification number on the test tube was recognizable only for the author of the thesis. All samples were destroyed after analysis. Data was stored and analyzed in a completely anonymous way, in accordance with the NTNU data storage guide (NTNU, 2022). Individual childcare centers, group units and children are not recognizable in the research reports.

It has to be noted that toddlers were not able to be informed of and consent to the participation and saliva sampling in our studies. However, this does not mean that we should exclude young children from research. According to the United Nations Convention on the Rights of the Child (UNCRC), children have a right to be heard in research (Alderson & Morrow, 2011) and this includes large quantitative studies. Given the likely validity of cortisol levels as an indicator of stress, parents' informed consent, respect for children's refusal during sampling procedures, anonymous sample storage and analysis, as well as a subsequent destruction of samples, I argue that measurement of cortisol levels is one appropriate way of including very young children's perspective into large quantitative research studies. The children in our studies did not profit directly from their participation, however our investigation was still highly relevant for their age group and hopefully our findings will contribute to better childcare provisions for toddlers in the future (Alderson & Morrow, 2011).

Strengths

Our studies possess some unique strengths. Whenever possible, we took measurements across two days to account for intraindividual variations of cortisol levels. We have a

comparably high number of participants, especially in our second study. In addition, we have measured cortisol levels in the evening on most occasions, which was only done by a few studies previously. Our third study is to the best of my knowledge the first to follow toddlers' stress levels longitudinally through a year in childcare. In addition, we only applied adequately validated and recognized measurement tools in our data collection. Furthermore, we integrated children's perspectives into research by measuring their cortisol levels and thereby included them as informants alongside parents and caregivers.

Limitations

The findings of our studies have to be viewed in the light of certain limitations. Our sample had a low socioeconomic risk with an educational level and income above the Norwegian average. Further, only very few children had a non-Norwegian language background. There is little research on the childcare stress of children with a high socioeconomic risk. Disadvantaged preschoolers have been observed to show higher cortisol levels in childcare compared to middle-class peers (Eckstein-Madry et al., 2021). Berry et al. (2014) on the other hand found more weekly hours of early childcare attendance to be predictive of lower cortisol levels at age four in children with a high socioeconomic risk, while the opposite was observed for children from a low-risk background. Hence, there is reason to believe that children with a high socioeconomic risk may show differ in their stress regulation and our findings are likely only applicable for a middle-class population. We were furthermore not able to collect data on the quality of caregiving in families and children's attachment style. Insecurely attached children have for example been observed to be more stressed during acclimatization with mothers present in the childcare center (Ahnert et al., 2004). Measuring these factors and investigating their relation to stress levels could have provided us with more insight. Also, we had no control group of children

who did not attend childcare. However, a stay-at-home control group would have been difficult to recruit and would have offered only limited comparability. The vast majority of toddlers attends childcare in Norway (Statistics Norway, 2022e) and families who do not enroll their children in childcare differ systematically from the families participating in our study (Statistics Norway, 2019).

A weak link when it comes to the reliability of our data lays in the adherence to the sampling protocol. There may have been deviation from the sampling time schedule and there was no data on children's medication intake, wake-up times, nap times and mealtimes on the days of saliva sampling (Hanrahan et al., 2006). The Thrive by Three study had a high participant burden with frequent saliva sampling, extensive questionnaires, and a quality building program. We chose therefore to refrain from asking caregivers and parents additional questions.

Parents and caregivers had precise instructions on when and how to take samples and how to store them. However, samples may have been taken slightly earlier or later than instructed. We accepted samples that were taken in a time window of one hour before and one hour after the set time point. Imprecisions when it comes to sampling time may account for some of the variability in cortisol levels. Ensuring a stricter adherence to the sampling schedule or recording sample times could certainly have increased the reliability of measurements and led to a more precise picture of toddlers' stress in childcare. There may also have been failures to freeze samples, however this, as well as several freeze and thaw cycles, should not influence cortisol concentration in saliva (Garde & Hansen, 2005). Samples were collected timely from childcare centers, and I therefore assume that delays in freezing samples had most likely only very little impact on our data. We have not controlled for medication intake. Many medications potentially influence HPA activity or cross-react with antibodies during immunoassay (Granger

et al., 2009). However, we do not expect systematic and widespread use of medications by toddlers. The most likely influence may have been a contamination of our samples with corticosteroids (e.g., used against eczema and allergies) (Granger et al., 2009). Measurements of above 30 nmol/l were therefore excluded. Most of the excluded measurements had a highly improbable value and I am therefore fairly confident that contamination could mostly be identified, and that medication intake should not have a systematic impact on the data. Nevertheless, I cannot rule out that there were individual samples which were compromised by medication intake. Intake of certain foods may alter the characteristics of saliva or cause a surge in cortisol levels (Hanrahan et al., 2006). I assume that the time points for meals are similar in Norwegian center care. Morning samples were taken in good time after breakfast and we advised caregivers to take afternoon samples just before snack time, when children were already seated at the table. However, we cannot exclude that some afternoon samples were taken right after eating and this may affect the reliability of our measurements to some degree. Furthermore, waking up from sleep is commonly causing a surge in cortisol levels, termed cortisol awakening response (CAR) (Mesas et al., 2022). Again, I assume that there is a great similarity in the day rhythm across Norwegian childcare centers and that most children likely had spent some hours already in the center by the time morning samples were taken. Morning cortisol levels were presumably not elevated by a cortisol awakening response. Afternoon samples on the other hand may have been taken closer to the midday nap and afternoon cortisol levels may therefore be somewhat elevated by a cortisol awakening response. However, we did not observe an afternoon cortisol elevation at home. A recent meta-analysis concluded that daytime napping may have a minor impact on young children's cortisol levels (Mesas et al., 2022). Yet, there may have been an influence of

cortisol awakening responses on cortisol levels, and controlling for sleep times would have enhanced the reliability of our measurements.

The link between temperament and stress was tested in a linear fashion and we did not account for potential non-linear relationships between stress and temperament according to other factors (e.g., childcare characteristics) (Belsky & Pluess, 2009; Ellis & Boyce, 2008).

4.5 Further research needs

We conducted, to the best of my knowledge, the until now only long-term investigation of toddlers' cortisol levels in childcare. More longitudinal research is therefore highly needed. Future studies should follow children from transition and preferably through both of their years of early childcare attendance to learn more about longitudinal profiles of childcare stress. With longitudinal measurements, there may be a higher likelihood of observing different profiles of stress activity in certain groups of children, compared to cross-sectional measurements. Children should be followed into the preschool and possibly elementary school years in order to investigate the effects of childcare stress. There is also a need to further investigate the link between stress and childcare quality (both at a group and a dyadic level), preferably with novel methods which take fluctuations of quality between dyads and situations into account. Further research also needs to study issues which the present work was not able to address, for example how childcare stress is linked to attachment style or non-linear relationships between stress and temperament. Future studies should also strive to recruit more diverse samples.

4.6 Conclusion

The studies included in the present thesis replicated the finding of afternoon cortisol elevations in toddlers in childcare. They also contributed new insights, including the following findings:

- The youngest children had higher afternoon stress levels after a month of childcare attendance. They may need longer time to adjust to childcare.
- Children in group-units with a lower caregiver availability and a lower degree of organizational flow were more stressed in childcare.
- The long-term pattern of stress activity split into two profiles according to well-being. Children with very high well-being may gradually habituate to childcare, while other children may become increasingly stressed over time.

In conclusion, the stress we observed in childcare was likely moderate and time-limited on average, yet it accumulated to some degree throughout the year.

It is unclear if afternoon cortisol elevations in childcare can be fully avoided for the toddler age group, but it should be an ambition to provide childcare practice which produces as little divergence from the diurnal cortisol rhythm as possible. Our studies and other research strongly suggest that childcare practice must be better adjusted to toddlers needs. There needs to be further effort from researchers, practitioners, and politicians to learn what low-stress childcare for toddlers looks like and subsequently, insights need to be put into practice. Guaranteeing at least one caregiver per three children during all opening hours, promoting high quality during the transition to childcare, and introducing subjects and programs specialized on toddlers' unique

needs into the education of childcare teachers would be appropriate first steps toward more toddler-friendly childcare provisions.

5 References

Abrahamsen, G. (2015). *Tilknytningsbaserte barnehager*. Universitetsforlaget.

Ahnert, L., Eckstein-Madry, T., Datler, W., Deichmann, F., & Piskernik, B. (2022). Stress during transition from home to public childcare. *Applied Developmental Science,* (advance online publication). https://doi.org/10.1080/10888691.2022.2070168

Ahnert, L., Gunnar, M. R., Lamb, M. E., & Barthel, M. (2004). Transition to child care: Associations with infant-mother attachment, infant negative emotion, and cortisol elevations. *Child Development, 75*(3), 639-650. https://doi.org/10.1111/j.1467-8624.2004.00698.x

Ahnert, L., Pinquart, M., & Lamb, M. E. (2006). Security of children's relationships with nonparental care providers: A meta-analysis. *Child Development, 77*(3), 664-679. https://doi.org/10.1111/j.1467-8624.2006.00896.x

Alderson, P., & Morrow, V. (2011). *The ethics of research with children and young people: A practical handbook*. SAGE Publications.

Alvestad, M., Gjems, L., Myrvang, E., Storli, J. B., Tungland, I. B. E., Velde, K. L., & Bjørnestad, E. (2019). *Kvalitet i barnehagen*. Universitetet i Stavanger.

Alvestad, T. (2010). *Barnehagens relasjonelle verden : små barn som kompetente aktører i produktive forhandlinger.* (294). Acta Universitatis Gothoburgensis.

Baumeister, D., Lightman, S. L., & Pariante, C. M. (2014). The interface of stress and the HPA axis in behavioural phenotypes of mental illness. In C. M. Pariante & M. D. Lapiz-Bluhm (Eds.), *Behavioral neurobiology of stress-related disorders* (pp. 13-24). Springer Berlin.

Belsky, J., & Pluess, M. (2009). The nature (and nurture?) of plasticity in early human development. *Perspectives on Psychological Science, 4*(4), 345-351. https://doi.org/10.1111/j.1745-6924.2009.01136.x

Bernard, K., Peloso, E., Laurenceau, J. P., Zhang, Z., & Dozier, M. (2015). Examining change in cortisol patterns during the 10-week transition to a new child-care setting. *Child Development, 86*(2), 456-471. https://doi.org/10.1111/cdev.12304

Berry, D., Blair, C., Ursache, A., Wiloughy, M., Garrett-Peters, P., Veron-Feagans, L., Bratsch Hines, M., Mills-Koonce, W. R., Granger, D. A. (2014). Child care and cortisol across early childhood: Context matters. *Developmental Psychology, 50*(2), 514-525. https://doi.org/10.1037/a0033379

Bjørnestad, E., & Os, E. (2018). Quality in Norwegian childcare for toddlers using ITERS-R. *European Early Childhood Education Research Journal, 26*(1), 111-127. https://doi.org/10.1080/1350293X.2018.1412051

Blair, C., Raver, C. C., Granger, D., Mills-Koonce, W. R., & Hibel, L. (2011). Allostasis and allostatic load in the context of poverty in early childhood. *Developmental Psychopathology, 23*(3), 845-857. https://doi.org/10.1017/S0954579411000344

Bradley, R. H., & Vandell, D. L. (2007). Child care and the well-being of children. *Archades of Pediatric Adolescene Medicine, 161*, 669-676. https://doi.org/10.1001/archpedi.161.7.669

Bratterud, Å., Sandseter, E. B. H., & Seland, M. (2012). *Barns trivsel og medvirkning i barnehagen. Barn, foreldre og ansattes perspektiver*. NTNU Samfunnsforskning.

Broekhuizen, M. L., Aken, M. A. G., Dubas, J. S., & Leseman, P. P. M. (2018). Child care quality and Dutch 2- and 3-year-olds' socio-emotional outcomes: Does the amount of care matter? *Infant and Child Development, 27*(1). https://doi.org/10.1002/icd.2043

Bronfenbrenner, U. (1979). *The ecology of human development: Experiments by nature and design*. Harvard University Press.

Brooker, L. (2008). *Supporting transitions in the early years*. McGraw-Hill Education.

Burchinal, M. (2018). Measuring early care and education quality. *Child Development Perspectives, 12*(1), 3-9. https://doi.org/10.1111/cdep.12260

Burger, K. (2010). How does early childhood care and education affect cognitive development? An international review of the effects of early interventions for children from different social backgrounds. *Early Childhood Research Quarterly, 25*(2), 140-165. https://doi.org/10.1016/j.ecresq.2009.11.001

Cadima, J., Aguiar, C., Guedes, C., Wysłowska, O., Salminen, J., Slot, P., Barata C. & Lerkkanen, M. K. (2022). Process quality in toddler classrooms in four European countries. *Early Education and Development,* (advance online publication). https://doi.org/10.1080/10409289.2022.2139548

Cadima, J., Barros, S., Nata, G., Coelho, V., & Barata, C. (2020). *Literature review on early childhood education and care for children under the age of 3*: OECD Publishing.

Camilli, G., Vargas, S., Ryan, S., & Barnett, W. S. (2010). Meta-analysis of the effects of early education interventions on cognitive and social development. *Teachers College Record, 112*(3), 579-620. https://doi.org/10.1177/016146811011200303

Center on the Developing Child. (2004a). Children's emotional development is built into the architecture of their brains. https://harvardcenter.wpenginepowered.com/wp-content/uploads/2004/04/Childrens-Emotional-Development-Is-Built-into-the-Architecture-of-Their-Brains.pdf

Center on the Developing Child. (2004b). Young children develop in an environment of relationships. https://developingchild.harvard.edu/wp-content/uploads/2004/04/Young-Children-Develop-in-an-Environment-of-Relationships.pdf

Center on the Developing Child. (2007a). The science of early childhood development (InBrief). https://developingchild.harvard.edu/resources/inbrief-science-of-ecd/

Center on the Developing Child. (2007b). The timing and quality of early experiences combine to shape brain architecture. https://harvardcenter.wpenginepowered.com/wp-content/uploads/2007/05/Timing_Quality_Early_Experiences-1.pdf

Center on the Developing Child. (2010). Early experience can alter gene expression and affect long-term development. https://harvardcenter.wpenginepowered.com/wp-content/uploads/2010/05/Early-Experiences-Can-Alter-Gene-Expression-and-Affect-Long-Term-Development.pdf

Center on the Developing Child. (2014). Excessive stress disrupts the architecture of the developing brain. https://developingchild.harvard.edu/wp-content/uploads/2005/05/Stress_Disrupts_Architecture_Developing_Brain-1.pdf

Center on the Developing Child. (2017). Toxic stress. https://developingchild.harvard.edu/science/key-concepts/toxic-stress/

Center on the Developing Child. (2020). Connecting the brain to the best of the body: Early childhood development and lifelong health are deeply intertwined. https://developingchild.harvard.edu/resources/connecting-the-brain-to-the-rest-of-the-body-early-childhood-development-and-lifelong-health-are-deeply-intertwined/

Dalli, C. E., White, J., Rockel, J., Duhn, I., Buchanan, E., Davidson, S., Ganly, S., Kus, L., & Wang, B. (2011). *Quality early childhood education for under-two-year-olds: What should it look like? A literature review*. Ministry of Education New Zealand.

Datler, W., Datler, M., & Funder, A. (2010). Struggling against a feeling of becoming lost: a young boy's painful transition to day care. *Infant Observation, 13*(1), 65-87. https://doi.org/10.1080/13698031003606659

De Schipper, E. J., Marianne Riksen-Walraven, J., & Geurts, S. A. E. (2006). Effects of child-caregiver ratio on the interactions between caregivers and children in child-care centers: An experimental study. *Child Development, 77*(4), 861-874. https://doi.org/10.1111/j.1467-8624.2006.00907.x

De Schipper, J. C., Tavecchio, L. W. C., Van Ijzendoorn, M. H., & Linting, M. (2003). The relation of flexible child care to quality of center day care and children's socio-emotional functioning: A survey and observational study. *Infant Behavior & Development, 26*(3), 300-325. https://doi.org/10.1016/S0163-6383(03)00033-X

De Schipper, J. C., Tavecchio, L. W. C., Van Ijzendoorn, M. H., & Van Zeijl, J. (2004). Goodness-of-fit in center day care: relations of temperament, stability, and quality of care with the child's adjustment. *Early Childhood Research Quarterly, 19*(2), 257-272. https://doi.org/10.1016/j.ecresq.2004.04.004

De Schipper, J. C., Van Ijzendoorn, M. H., & Tavecchio, L. W. C. (2004). Stability in center day care: Relations with children's well-being and problem behavior in day care. *Social Development, 13*, 531-550.

De Vet, S. M., Vrijhof, C. I., Van Der Veek, S. M. C., Linting, M., & Vermeer, H. J. (2023). Young children's cortisol levels at out-of-home child care: A meta-analysis. *Early Childhood Research Quarterly, 63*, 204-218. https://doi.org/10.1016/j.ecresq.2022.12.015

Degotardi, S., & Pearson, E. (2009). Relationship theory in the nursery: Attachment and beyond. *Contemporary Issues in Early Childhood, 10*(2), 144-155. https://doi.org/10.2304/ciec.2009.10.2.144

Del Giudice, M., Ellis, B. J., & Shirtcliff, E. A. (2011). The adaptive calibration model of stress responsivity. *Neuroscience and Biobehavioral Reviews, 35*(7), 1562-1592. https://doi.org/10.1016/j.neubiorev.2010.11.007

Deynoot-Schaub, M. G., & Riksen-Walraven, J. M. (2006). Peer interaction in child care centres at 15 and 23 months: Stability and links with children's socio-emotional adjustment. *Infant Behavior & Development, 29*(2), 276-288. https://doi.org/10.1016/j.infbeh.2005.12.005

Deynoot-Schaub, M. J. G., & Riksen-Walraven, J. M. (2006). Peer contacts of 15-month-olds in childcare: Links with child temperament, parent-child interaction and quality of childcare. *Social Development, 15*(4), 709-729. https://doi.org/10.1111/j.1467-9507.2006.00366.x

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses. A theoretical integration an synthesis of laboratory research. *Psychological Bulletin, 130*(3), 355-391. https://doi.org/10.1037/0033-2909.130.3.355

Dressendörfer, R. A., Kirschbaum, C., Rohde, W., Stahl, F., & Strasburger, C. (1992). Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary

cortisol measurement. *Journal of Steroid Biochemistry and Molecular Biology, 43*(7), 683-692. https://doi.org/10.1016/0960-0760(92)90294-S

Drugli, M. B. (2017). *Liten i barnehagen*. Cappelen Damm akademisk.

Drugli, M. B. (2020). *Delrapport for folkehelseprosjektet "Liten og ny i barnehagen". Evaluering av trinn 2*. Regionalt kunnskapssenter for barn og unge - psykisk helse og barnevern, NTNU.

Drugli, M. B., Grip, M. S., & Solheim, E. (2017). Rutiner for tilvenning i norske barnehager. *Barnehagefolk, 1*, 92-98.

Drugli, M. B., Nystad, K., Lydersen, S., & Brenne, A. S. (2023). Do toddlers' levels of cortisol and the perceptions of parents and professional caregivers tell the same story about transition from home to childcare?: A mixed method study. *Frontiers in Psychology,* (Accepted for publication).

Drugli, M. B., Solheim, E., Lydersen, S., Moe, V., Smith, L., & Berg-Nielsen, T. S. (2018). Elevated cortisol levels in Norwegian toddlers in childcare. *Early Child Development and Care, 188*(12), 1684-1695. https://doi.org/10.1080/03004430.2016.1278368

Ebbeck, M., & Yim, H. Y. B. (2009). Rethinking attachment: fostering positive relationships between infants, toddlers and their primary caregivers. *Early Child Development and Care, 179*(7), 899-909. https://doi.org/10.1080/03004430701567934

Eckstein-Madry, T., Piskernik, B., & Ahnert, L. (2021). Attachment and stress regulation in socioeconomically disadvantaged children: Can public childcare compensate? *Infant Mental Health Journal, 42*(6), 839-850. https://doi.org/10.1002/imhj.21878

Elfer, P., Goldschmied, E., & Selleck, D. (2012). *Key persons in the early years. Building relationships for quality provisions in early years settings and primary schools.* Routledge.

Ellis, B. J., & Boyce, W. T. (2008). Biological sensitivity to context. *Current Directions in Psychological Science, 17*(3), 183-187. https://doi.org/10.1111/j.1467-8721.2008.00571.x

Ellis, B. J., & Boyce, W. T. (2011). Differential susceptibility to the environment: Toward an understanding of sensitivity to developmental experiences and context. *Developmental Psychopathology, 23*(1), 1-5. https://doi.org/10.1017/S095457941000060X

Engdahl, I. (2011). Toddler interaction during play in the Swedish preschool. *Early Child Development and Care, 181*(10), 1421-1439. https://doi.org/10.1080/03004430.2010.533269

Engel, A., Barnett, S. W., Anders, Y., & Taguma, M. (2015). *Early Childhood Education and Care Policy Review: Norway*. OECD Publishing.

Engel, M. L., & Gunnar, M. R. (2020). The development of stress reactivity and regulation during human development. *International Review of Neurobiology, 150*, 41-76. https://doi.org/10.1016/bs.irn.2019.11.003

Everly, G. S., Jr., & Lating, J. M. (2019). *A clinical guide to the treatment of the human stress response*. Springer.

Fabian, H., & Dunlop, A. W. (2007). *Outcomes of good practice in transition processes for children entering primary school. Working Papers in Early Childhood Development, No. 42.* Bernard Van Leer Foundation.

Fukkink, R. G. (2022). Exploring children's well-being in daycare: how do children feel all day? *European Early Childhood Education Research Journal, 30*(5), 730-744. https://doi.org/10.1080/1350293X.2021.2007971

Garde, A. H., & Hansen, Å. M. (2005). Long-term stability of salivary cortisol. *Scandinavian Journal of Clinical and Laboratory Investigation, 65*(5), 433-436. https://doi.org/10.1080/00365510510025773

Geoffroy, M.-C., Côté, S. M., Parent, S., & Séguin, J. R. (2006). Daycare attendance, stress, and mental health. *The Canadian Journal of Psychiatry, 51*(9), 607-615. https://doi.org/10.1177/070674370605100909

Gevers Deynoot-Schaub, M. J. J. M., & Riksen-Walraven, J. M. (2008). Infants in group care: Their interactions with professional caregivers and parents across the second year of life. *Infant Behavior & Development, 31*(2), 181-189. https://doi.org/10.1016/j.infbeh.2007.10.010

Gloeckler, L., & Cassell, J. (2012). Teacher practices with toddlers during social problem solving opportunities. *Early Childhood Education Journal, 40*(4), 251-257. https://doi.org/10.1007/s10643-011-0495-4

Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N., & Umeoka, E. H. d. L. (2018). A comprehensive overview on stress neurobiology: Basic concepts and clinical implications. *Frontiers in Behavioral Neuroscience, 12*, 127-127. https://doi.org/10.3389/fnbeh.2018.00127

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., Nugent, T. F., Herman, D. H., Clasen, L. S., Toga. A. W., Rapoport, J. L., & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through

early adulthood. *Proceedings of the National Academy of Science of the United States of America, 101*(21), 8174-8179. https://doi.org/10.1073/pnas.0402680101

Granger, D. A., Hibel, L. C., Fortunato, C. K., & Kapelewski, C. H. (2009). Medication effects on salivary cortisol: Tactics and strategy to minimize impact in behavioral and developmental science. *Psychoneuroendocrinology, 34*(10), 1437-1448. https://doi.org/10.1016/j.psyneuen.2009.06.017

Groeneveld, M. G., Vermeer, H. J., Van Ijzendoorn, M. H., & Linting, M. (2010). Children's wellbeing and cortisol levels in home-based and center-based childcare. *Early Childhood Research Quarterly, 25*(4), 502-514. https://doi.org/10.1016/j.ecresq.2009.12.004

Guerrero-Rosada, P., Weiland, C., McCormick, M., Hsueh, J., Sachs, J., Snow, C., & Maier, M. (2021). Null relations between CLASS scores and gains in children's language, math, and executive function skills: A replication and extension study. *Early Childhood Research Quarterly, 54*, 1-12. https://doi.org/10.1016/j.ecresq.2020.07.009

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology, 27*(1), 199-220. https://doi.org/10.1016/S0306-4530(01)00045-2

Gunnar, M. R., & Herrera, A. M. (2013). The development of stress reactivity: A neurobiological perspective. In P. D. Zelazo (Ed.), *The Oxford handbook of developmental psychology. Vol. 2: Self and other.* (pp 45-80). Oxford University Press.

Gunnar, M. R., & Vazquez, D. M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Developmental Psychopathology, 13*(3), 515-538. https://doi.org/10.1017/S0954579401003066

Hagström, B. (2010). *Kompletterande anknytningsperson på förskolan.* Doctoral Dissertation in Education: Malmö högskolan.

Hanrahan, K., McCarthy, A. M., Kleiber, C., Lutgendorf, S., & Tsalikian, E. (2006). Strategies for salivary cortisol collection and analysis in research with children. *Applied Nursing Research, 19*(2), 95-101. https://doi.org/10.1016/j.apnr.2006.02.001

Hayes, N., O'Toole, L., & Halpenny, A. M. (2017). *Introducing Bronfenbrenner: A Guide for Practitioners and Students in Early Years Education*. Taylor and Francis.

Iluz, R., Adi-Japha, E., & Klein, P. S. (2016). Identifying child-staff ratios that promote peer skills in child care. *Early Education and Development, 27*(7), 1077-1098. https://doi.org/10.1080/10409289.2016.1175240

Jebsen, J. K., & Norge. (2020). *Barnehageloven og forskrifter : med forarbeider og tolkninger 2020*. Pedlex.

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology, 19*, 313-333. https://doi.org/10.1016/0306-4530(94)90013-2

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test' -- a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*(1-2), 76-81. https://doi.org/10.1159/000119004

Kirschbaum, C., Steyer, R., Eid, M., Patalla, U., Schwenkmezger, P., & Hellhammer, D. (1990). Cortisol and behavior: 2. Application of a latent state-trait model to salivary cortisol. *Psychoneuroendocrinology, 15*(4), 297-307. https://doi.org/10.1016/0306-4530(90)90080-S

Klette, T., Drugli, M. B., & Aandahl, A. M. (2018). Together and alone. A study of interactions between toddlers and childcare providers during mealtime in Norwegian childcare centres. *Early Child Development and Care, 188*(3), 387-398. https://doi.org/10.1080/03004430.2016.1220943

Knudsen, E. I., Heckman, J. J., Cameron, J. L., & Shonkoff, J. P. (2006). Economic, neurobiological, and behavioral perspectives on building America's future workforce. *Proceedings of the National Academy of Science of the United States of America, 103*(27), 10155-10162. https://doi.org/10.1073/pnas.0600888103

Kontos, S., & Wachs, T. (2000). *Life in Early Childhood Programs Scale* (unpublished manuscript). Department of Child Development and Family Studies. Purdue University.

Koss, K. J., & Gunnar, M. R. (2018). Annual research review: Early adversity, the hypothalamic–pituitary–adrenocortical axis, and child psychopathology. *Journal of Child Psychology and Psychiatry, 59*(4), 327-346. https://doi.org/10.1111/jcpp.12784

Krueger, C., & Tian, L. (2004). A comparison of the general linear mixed model and repeated measures ANOVA using a dataset with multiple missing data points. *Biological Research for Nursing, 6*(2), 151-157. https://doi.org/10.1177/1099800404267682

La Paro, K. M., & Gloeckler, L. (2015). The context of child care for toddlers: The "Experience Expectable Environment". *Early Childhood Education Journal, 44*(2), 147-153. https://doi.org/10.1007/s10643-015-0699-0

La Paro, K. M., Hamre, B. K., & Pianta, R. C. (2012). *The Classroom Assessment Scoring System. Toddler Manual.* Brookes Publishing.

Legendre, A. (2003). Environmental features influencing toddlers' bioemotional reactions in day care centers. *Environment and Behavior, 35*, 523-549. https://doi.org/10.1177/0013916503035004005

Lekhal, R. (2012). Do type of childcare and age of entry predict behavior problems during early childhood? Results from a large Norwegian longitudinal study. *International Journal of Behavioral Development, 36*(3), 197-204. https://doi.org/10.1177/0165025411431409

Lekhal, R., Drugli, M. B., Berg-Nielsen, T. S., & Buøen, E. S. (2020). A model of intervention and implementation of quality building and quality control in childcare centers to strengthen the mental health and development of 1-3-year olds: Protocol for a randomized controlled trial of thrive by three. *JMIR Research Protocols, 9*(10), e17726-e17726. https://doi.org/10.2196/17726

Lekhal, R., Vartun, M., Gustavson, K., Helland, S. S., Wang, M. V., & Schjølberg, S. (2013). *Variasjon i barnehagekvalitet : den norske mor og barn-undersøkelsen : beskrivelser fra første datainnsamling fra barnehagene*. Nasjonalt folkehelseinstitutt.

Lindfors, P., & Lundberg, U. (2002). Is low cortisol release an indicator of positive health? *Stress and Health, 18*(4), 153-160. https://doi.org/10.1002/smi.942

Loman, M. M., & Gunnar, M. R. (2010). Early experience and the development of stress reactivity and regulation in children. *Neuroscience and Biobehavioral Reviews, 34*(6), 867-876. https://doi.org/10.1016/j.neubiorev.2009.05.007

Løkken, I. M., Bjørnestad, E., Broekhuizen, M. L., & Moser, T. (2018). The relationship between structural factors and interaction quality in Norwegian ECEC for toddlers. *International Journal of Child Care and Education Policy, 12*(1), 1-15. https://doi.org/10.1186/s40723-018-0048-z

Løkås, M. T. (2023). Politikerne snur om tilvenning - vil innføre "Liten og ny i barnehagen". https://www.barnehage.no/liten-og-ny-may-britt-drugli-tilvenning/politikerne-snur-om-tilvenning-vil-innfore-liten-og-ny-i-barnehagen/240767

Markström, A.-M., & Simonsson, M. (2017). Introduction to preschool: strategies for managing the gap between home and preschool. *Nordic Journal of Studies in Educational Policy, 3*(2), 179-188. https://doi.org/10.1080/20020317.2017.1337464

Mathiesen, K. S., & Tambs, K. (1999). The EAS Temperament Questionnaire—Factor structure, age trends, reliability, and stability in a Norwegian sample. *Journal of Child Psychology and Psychiatry, 40*(3), 431-439. https://doi.org/10.1111/1469-7610.00460

Mesas, A. E., Sánchez-López, M., Pozuelo-Carrascosa, D. P., Sequí-Domínguez, I., Jiménez-López, E., & Martínez-Vizcaíno, V. (2022). The role of daytime napping on salivary cortisol in children aged 0–5 years: a systematic review and meta-analysis. *European Journal of Pediatrics, 181*(4), 1437-1448. https://doi.org/10.1007/s00431-021-04371-x

NICHD - Early Child Care Research Network. (2002). Child-care structure -> process -> outcome: Direct and indirect effects of child-care quality on young children's development. *Psychological Science, 13*(3), 199-206. https://doi.org/10.1111/1467-9280.00438

NICHD Early Child Care Research Network. (2005). *Child care and child development : results from the NICHD study of early child care and youth development*. Guilford Press.

Norwegian Directorate for Education and Training. (2017). Framework Plan for Kindergartens. Content and Tasks. https://www.udir.no/globalassets/filer/barnehage/rammeplan/framework-plan-for-kindergartens2-2017.pdf

NTNU. (2022). Data storage guide. https://i.ntnu.no/wiki/-/wiki/English/Data+storage+guide

O'Connor, A. (2013). *Understanding Transitions in the Early Years. Supporting change through attachment and resilience.* Routledge.

OECD. (2017). *Starting strong 2017: Key OECD indicators on early childhood education and care*. https://read.oecd-ilibrary.org/education/starting-strong-2017_9789264276116-en

OECD. (2020). *Education at a Glance 2020: OECD Indicators*. OECD Publishing.

Orri, M., Boivin, M., Chen, C., Ahun, M. N., Geoffroy, M.-C., Ouellet-Morin, I., Tremblay, R. E., & Côté, S. M. (2021). Cohort profile: Quebec Longitudinal Study of Child Development (QLSCD). *Social Psychiatry and Psychiatric Epidemiology, 56*(5), 883-894. https://doi.org/10.1007/s00127-020-01972-z

Os, E. (2013). Opportunities knock: Mediation of peer-relations during meal-time in toddler groups. *Nordisk Barnehageforskning, 6*. https://doi.org/10.7577/nbf.460

Os, E., & Hernes, L. (2019). Children under the age of three in Norwegian childcare: Searching for qualities. In S. Garvis, H. Harju-Luukkainen, S. Sheridan, & P. Williams (Eds.), *Nordic Families, Children and Early Childhood Education* (pp. 139-171). Palgrave Macmillan.

Oslo kommune. (2022). *Bemanning i barnehager. Rapport 4.* Kommunerevisjonen.

Ouellet-Morin, I., Tremblay, R. E., Boivin, M., Meaney, M., Kramer, M., & Cote, S. M. (2010). Diurnal cortisol secretion at home and in child care: A prospective study of 2-year-old toddlers. *Journal of Child Psychology and Psychiatry, 51*(3), 295-303. https://doi.org/10.1111/j.1469-7610.2009.02167.x

Phillips, D. A., Fox, N. A., & Gunnar, M. R. (2011). Same place, different experiences: Bringing individual differences to research in child care. *Child Development Perspectives, 5*(1), 44-49. https://doi.org/10.1111/j.1750-8606.2010.00155.x

Pianta, R. C., Downer, J., & Hamre, B. (2016). Quality in early education classrooms: Definitions, gaps, and systems. *Future of Children, 26*(2), 119-137. https://doi.org/10.1353/foc.2016.0015

Pianta, R. C., Hamre, B., & Stuhlman, M. (2003). Relationships between teachers and children. In W. M. Reynolds & G. E. Miller (Eds.), *Handbook of psychology: Educational psychology, Vol. 7,* (pp. 199–234). John Wiley & Sons.

Pianta, R. C., La Paro, K. M., & Hamre, B. K. (2008). *Classroom Assessment Scoring System. Pre-K Manual*. Brookes Publishing.

Salimetrics. (2019). Collection method: SalivaBio Children's Swab (SCS). https://salimetrics.com/wp-content/uploads/2018/02/children-swab-saliva-collection-instructions.pdf

Sammons, P. (2010). Does pre-school make a difference? Identifying the impact of pre-school on children's cognitive and social behavioural development at different ages. In K. Sylva, E. Melhuish, P. Sammons, Sirah-Blatchford, & B. Taggart (Eds.), *Early childhood matters: evidence from the effective pre-school and primary education project* (pp. 92-113). Routledge.

Saridjan, N. S., Velders, F. P., Jaddoe, V. W. V., Hofman, A., Verhulst, F. C., & Tiemeier, H. (2014). The longitudinal association of the diurnal cortisol rhythm with internalizing and externalizing problems in pre-schoolers. The Generation R Study. *Psychoneuroendocrinology, 50*, 118-129. https://doi.org/10.1016/j.psyneuen.2014.08.008

Schore, J., & Schore, A. (2008). Modern attachment theory: The central role of affect regulation in development and treatment. *Clinical Social Work Journal, 36*(1), 9-20. https://doi.org/10.1007/s10615-007-0111-7

Seland, M., Beate Hansen Sandseter, E., & Bratterud, Å. (2015). One- to three-year-old children's experience of subjective wellbeing in day care. *Contemporary Issues in Early Childhood, 16*(1), 70-83. https://doi.org/10.1177/1463949114567272

Shaffer, D. R., & Kipp, K. (2014). *Developmental psychology : childhood and adolescence (9th ed.)*.Wadsworth Cengage Learning.

Simons, S. S. H., Beijers, R., Cillessen, A. H. N., & Weerth, C. D. (2015). Development of the cortisol circadian rhythm in the light of stress early in life. *Psychoneuroendocrinology, 62*, 292-300. https://doi.org/10.1016/j.psyneuen.2015.08.024

Singer, E., Van Hoogdalem, A.-G., De Haan, D., & Bekkema, N. (2012). Day care experiences and the development of conflict strategies in young children. *Early Child Development and Care, 182*(12), 1661-1672. https://doi.org/10.1080/03004430.2011.640753

Slot, P. L., & Bleses, D. (2018). Individual children's interactions with teachers, peers, and tasks: The applicability of the inCLASS Pre-K in Danish preschools. *Learning and Individual Differences, 61*, 68-76. https://doi.org/10.1016/j.lindif.2017.11.003

Slot, P. L., Boom, J., Verhagen, J., & Leseman, P. P. M. (2017). Measurement properties of the CLASS Toddler in ECEC in The Netherlands. *Journal of Applied Developmental Psychology, 48*, 79-91. https://doi.org/10.1016/j.appdev.2016.11.008

Slot, P. L., Leseman, P. P. M., Verhagen, J., & Mulder, H. (2015). Associations between structural quality aspects and process quality in Dutch early childhood education and care

settings. *Early Childhood Research Quarterly, 33*, 64-76. https://doi.org/10.1016/j.ecresq.2015.06.001

Statistics Norway. (2019). Laveste andel mottakere på 20 år. https://www.ssb.no/sosiale-forhold-og-kriminalitet/artikler-og-publikasjoner/laveste-andel-mottakere-pa-20-ar

Statistics Norway. (2022a). Educational attainment of the population. https://www.ssb.no/en/utdanning/utdanningsniva/statistikk/befolkningens-utdanningsniva

Statistics Norway. (2022b). Families and households. https://www.ssb.no/en/befolkning/barn-familier-og-husholdninger/statistikk/familier-og-husholdninger

Statistics Norway. (2022c). Immigrants and Norwegian-born to immigrant parents https://www.ssb.no/en/befolkning/innvandrere/statistikk/innvandrere-og-norskfodte-med-innvandrerforeldre

Statistics Norway. (2022d). Income and wealth statistics for households. Retrieved from https://www.ssb.no/en/inntekt-og-forbruk/inntekt-og-formue/statistikk/inntekts-og-formuesstatistikk-for-husholdninger

Statistics Norway. (2022e). Kindergartens. https://www.ssb.no/en/utdanning/statistikker/barnehager

Suhonen, E., Sjaniemi, N. K., Alijoki, A., & Nislin, M. A. (2018). Children's biological givens, stress responses, language and cognitive abilities and family background after entering kindergarten in toddlerhood. *Early Child Development and Care, 188*(3), 345-358. https://doi.org/10.1080/03004430.2016.1218157

Sumner, M. M., Bernard, K., & Dozier, M. (2010). Young Children's Full-Day Pattern of Cortisol Production on Child Care Days. *Archives of Psychiatric & Adolescent Medicine, 164*(6), 567-571. https://doi.org/10.1001/archpediatrics.2010.85

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 & Adolescent Psychiatry, 29*(9), 1217-1229. https://doi.org/10.1007/s00787-019-01432-3

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. *Developmental Psychobiology, 63*(S1), https://doi.org/10.1002/dev.22223

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. https://doi.org/10.1111/cdev.13435

Thoresen, M., & Gjessing, H. K. (2012). Mixed models. In M. B. Veierød, S. Lydersen, & P. Laake (Eds.), *Medical Statistics in clinical and epidemiological research* (pp. 231-258). Gyldendal Akademisk.

Undheim, A. M., & Drugli, M. B. (2012). Perspective of parents and caregivers on the influence of full-time day-care attendance on young children. *Early Child Development and Care, 182*(2), 233-247. https://doi.org/10.1080/03004430.2011.553678

Utdanningsdirektoratet. (2021). Barn, barnehager og ansatte. https://www.udir.no/tall-og-forskning/statistikk/statistikk-barnehage/analyser/fakta-om-barnehager/tall-om-barnehagen/#:~:text=Selv%20om%209%20er%20vanligste,enn%2018%20barn%20i%20storebarnsgruppene.

Van Schaik, S. D. M., Leseman, P. P. M., & De Haan, M. J. (2018). Using a group-centered approach to observe interactions in early childhood education. *Child Development, 89*(3), 897-913. https://doi.org/10.1111/cdev.12814

Van Trijp, C. P. J. (2023). *Toddlers' social-wmotional well-being in early childhood education and care: The role of child temperament and process quality. Dissertation for the degree of PhD*. Oslo University.

Van Trijp, C. P. J., Lekhal, R., Drugli, M. B., Rydland, V., & Solheim Buøen, E. (2021). Validation of the Leiden Inventory for the Child's Well-Being in Daycare (LICW-D) Questionnaire in Norwegian early childhood education and care centers. *Frontiers in Psychology, 12*. https://doi.org/10.3389/fpsyg.2021.767137

Van Trijp, C. P. J., Lekhal, R., Drugli, M. B., Rydland, V., Van Gils, S., Vermeer, H. J., & Buøen, E. S. (2021). The association between toddlers' temperament and well-being in Norwegian early childhood education and care, and the moderating effect of center-based daycare process quality. *Frontiers in Psychology, 12*. https://doi.org/https://doi.org/10.3389/fpsyg.2021.763682

Vermeer, H. J., & Groeneveld, M. G. (2017). Children's physiological responses to childcare. *Current Opinion in Psychology, 15*, 201-206. https://doi.org/10.1016/j.copsyc.2017.03.006

Vermeer, H. J., Groeneveld, M. G., Larrea, I., Van Ijzendoorn, M. H., Barandiaran, A., & Linting, M. (2010). Child care quality and children's cortisol in Basque country and the Netherlands. *Journal of Applied Developmental Psychology, 31*(4), 339-347. https://doi.org/10.1016/j.appdev.2010.05.001

Vermeer, H. J., & Van Ijzendoorn, M. H. (2006). Children's elevated cortisol levels at daycare: A review and meta-analysis. *Early Childhood Research Quarterly, 21*(3), 390-401. https://doi.org/10.1016/j.ecresq.2006.07.004

Vors, O., Marqueste, T., & Mascret, N. (2018). The Trier Social Stress Test and the Trier Social Stress Test for Groups: Qualitative investigations. *PLoS One, 13*(4). https://doi.org/10.1371/journal.pone.0195722

Wachs, T. D., Gurkas, P., & Kontos, S. (2004). Predictors of preschool children's compliance behavior in early childhood classroom settings. *Journal of Applied Developmental Psychology, 25*(4), 439-457. https://doi.org/10.1016/j.appdev.2004.06.003

Watamura, S., Donzella, B., Alwin, J., & Gunnar, M. R. (2003). Morning-to afternoon increases in cortisol concentrations for infants and toddlers at child care: age differences and behavioral correlates. *Child Development, 74*(4), 1006-1020. https://doi.org/10.1111/1467-8624.00583

Watamura, S. E., Coe, C. L., Laudenslager, M. L., & Robertson, S. S. (2010). Child care setting affects salivary cortisol and antibody secretion in young children. *Psychoneuroendocrinology, 35*(8), 1156-1166. https://doi.org/10.1016/j.psyneuen.2010.02.001

Watamura, S. E., Donzella, B., Kertes, D., & Gunnar, M. (2004). Developmental changes in baseline cortisol activity in early childhood: Relations with napping and effortful control. *Developmental Psychobiology, 45*(3), 125-133. https://doi.org/10.1002/dev.20026

Weiland, C., Moffett, L., Rosada, P. G., Weissman, A., Zhang, K., Maier, M., Snow, C., McCormick, M., Hsueh, J., & Sachs, J. (2023). Learning experiences vary across young children in the same classroom: evidence from the individualizing student instruction

measure in the Boston public schools. *Early Childhood Research Quarterly, 63*, 313-326. https://doi.org/10.1016/j.ecresq.2022.11.008

Wu, J., Phillip, T.-M., Doretto, V., Van Noordt, S., Chaplin, T. M., Hommer, R. E., Mayes, L. C., & Crowley, M. J. (2019). An inactive control of the 'Trier Social Stress Test' for youth 10–17 years: Neuroendocrine, cardiac, and subjective responses. *Psychoneuroendocrinology, 104*, 152-164. https://doi.org/10.1016/j.psyneuen.2019.02.027

6 Appendix

Paper 1

European Early Childhood Education Research Journal

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/recr20

Toddlers' stress during transition to childcare

K. Nystad, M. B. Drugli, S. Lydersen, R. Lekhal & E. S. Buøen

To cite this article: K. Nystad, M. B. Drugli, S. Lydersen, R. Lekhal & E. S. Buøen (2021): Toddlers' stress during transition to childcare, European Early Childhood Education Research Journal, DOI: 10.1080/1350293X.2021.1895269

To link to this article: https://doi.org/10.1080/1350293X.2021.1895269

Published online: 23 Mar 2021.

EUROPEAN EARLY CHILDHOOD EDUCATION RESEARCH JOURNAL
https://doi.org/10.1080/1350293X.2021.1895269

Toddlers' stress during transition to childcare

K. Nystad [a], M. B. Drugli [a,b], S. Lydersen [a], R. Lekhal [c,d] and E. S. Buøen [c,e]

[a]Regional Centre for Child and Youth Mental Health and Child Welfare, Faculty of Medicine and Health Science, Department of Mental Health, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; [b]Centre of the Study of Educational Practice, Inland Norway University of Applied Sciences, Elverum, Norway; [c]Department of Communication and Culture, BI Norwegian Business School, Oslo, Norway; [d]Department of Education, University of Oslo, Oslo, Norway; [e]Regional Centre for Child and Adolescent Mental Health Eastern and Southern Norway, Oslo, Norway

ABSTRACT

In toddlers, the transition from home to childcare might elicit high levels of the stress hormone cortisol. Measuring cortisol may give an indicator for children's experience and hence, may help improve this transition. We applied linear mixed model analyses to investigate the cortisol levels of 119 toddlers during their transition to childcare across time of day (morning, afternoon, and evening) and phase (accompanied by parents, separated from parents, and after four to six weeks in childcare). The influence of age, gender, number of siblings, and childcare group size was analyzed. Time of day and phase influenced cortisol levels significantly. On average, children had elevated cortisol levels in the afternoon throughout transition, with the peak coming in the separation phase. Cortisol levels declined significantly toward the evening. Children younger than 14 months showed higher evening levels and higher afternoon levels after 4–6 weeks in childcare. The findings suggest that the onset of childcare – particularly separation from parents – may be demanding for toddlers. Low evening levels indicate relief of tension at home. Higher levels of afternoon cortisol of under 14-months-old children at the follow-up measurement may indicate that younger children need more time to settle in at childcare.

KEYWORDS

Toddler; cortisol; childcare; transition; stress; Thrive by Three

Introduction

An increasing number of toddlers (one- and two-year-old children) in OECD countries are being enrolled in an out-of-home daycare arrangement (Organisation for Economic Co-operation and Development 2017). In Norway, more than 80% of one- and two-year-old children attend childcare (Statistics Norway 2020). Increased early childcare attendance has sparked debates about the effects of childcare on children's development and attachment relationships (Belsky 2001). Research, though, cannot connect early childcare to systematically adverse outcomes (Lekhal 2012; NICHD - Early Child Care Research

CONTACT Kathrin Nystad kathrin.nystad@ntnu.no Regional Centre for Child and Youth Mental Health and Child Welfare, Norwegian University of Science and Technology (NTNU), P.O. Box 8905, MTFS, N-7491 Trondheim, Norway

Network 2002). Yet issues of childcare concern a large percentage of young children today and entering childcare may be a demanding process for many toddlers. Transition practices have recently been discussed among parents, caregivers, and researchers in Norway, particularly parents' important role during acclimatization (Drugli 2017; Humberset and Andersen 2018; Ramberg 2017).

The transition from home to childcare can be particularly demanding for toddlers. In childcare, toddlers meet new adults and peers in an unfamiliar environment. For the first time, many toddlers spend significant parts of the day without their parents, on whom they rely for emotional regulation (Ereky-Stevens et al. 2018). Observational studies have found that many children show signs of struggle during their first weeks in childcare (Datler et al. 2012; Datler, Datler, and Funder 2010). O'Connor (2013) and Brooker (2008) argue that good transitions are important for children's short- and long-term wellbeing, as well as their development in childcare. At the same time, we do not know enough about how toddlers react to transitions. There is little quantitative research on this matter.

Measuring the levels of the stress hormone cortisol may give an indication of children's experience in childcare, hence forming a method to research these transitions systematically (Vermeer and van Ijzendoorn 2006). Measuring cortisol levels can help in understanding a toddler's perception of transition to childcare in greater detail and may enable us to better adjust practices to meet children's needs. So far, few studies have looked at toddlers' cortisol activity in childcare.

The important first transition

Going from home to childcare is the first major transition in many children's lives. Toddlers may not be emotionally and cognitively mature enough to regulate their feelings during separation from their parents, and might therefore not be able to adjust to a new environment with little difficulty (Schore and Schore 2008).

Observational studies have detected signs of distress (Cryer et al. 2005; Datler et al. 2012) and the tendency for staff to overlook children's silent struggles during transitions (Datler et al. 2012; Datler, Datler, and Funder 2010). However, toddlers have also been observed navigating transitions in an active manner and without signs of greater distress (Simonsson 2015). Qualitative findings have suggested that a gradual acclimatization, one with a high involvement of parents and a primary key person among the caregivers, eases a transition (Brooker 2008; Ebbeck and Yim 2009; Markström and Simonsson 2017; Undheim and Drugli 2012).

While transitions require adjustment and may evoke feelings of insecurity, they also hold great opportunity to acquire new competences and relationships if done well (Brooker 2008). According to Bronfenbrenner's (1979) ecological systems theory, the transition from a familiar micro-system (the family) to an unfamiliar one (the childcare center) can be facilitated by a parent entering the new system together with the child as a bridge-builder. A good connection between the micro-systems is of great importance for the child. If a parent is present in the new setting and has the opportunity to interact positively together with the child and the new caregiver, a row of benefits, such as greater security and new social relationships, can emerge. In Reggio Emilia childcare centers in Italy, caregivers actively work to establish relationships with children and parents

during transition, 'Inserimento'. Parents are recognized as a central basis for children, from which they can get to know caregivers and the childcare center. Children's attention is gradually directed from parents toward the new caregivers. Ideally, caregivers, children and parents have met several times before childcare starts (Degotardi and Pearson 2014). The previously mentioned key person approach builds amongst other on attachment theory by Bowlby (Elfer, Goldschmied, and Selleck 2012). Young children seek to form close relationships. Such special relationships are important for children to regulate feelings and develop emotional competences (Schore and Schore 2008), which are an essential ingredient for healthy brain development (Center on the Developing Child 2012). Today, it is recognized that also professional caregivers can become important relational figures to a child. A key-person approach may help to establish close relationships to caregivers, giving children a safe haven in childcare and opportunity for meaningful interactions (Elfer, Goldschmied, and Selleck 2012).

Cortisol in childcare

Cortisol is the main product of the hypothalamic–pituitary–adrenal axis (HPA), one of the body's major stress systems. Cortisol is also part of regular metabolism. Cortisol levels generally vary in a diurnal cycle, with the highest values in the morning and a steady decline throughout the day (Gunnar and Herrera 2013). This typical pattern is established in humans from an early age (Gunnar and Quevedo 2007) and has been observed by Watamura et al. (2004) in children aged 12 months. Cortisol levels rise if one perceives a situation to be overly demanding and socially threatening (Gunnar and Quevedo 2007). Elevated levels of cortisol may be an indicator of social stress (Kirschbaum et al. 1990; Kirschbaum and Hellhammer 1994; Vermeer and van Ijzendoorn 2006), and hence, measuring cortisol may be a suitable method to evaluate children's experiences in childcare.

A meta-analysis by Vermeer and van Ijzendoorn (2006) suggested that toddlers tend to show higher levels of cortisol in childcare when compared with them being at home, and they do so more than other age groups. This finding has been supported in further studies (Bernard et al. 2015; Drugli et al. 2018; Groeneveld et al. 2010; Ouellet-Morin et al. 2010; Sumner, Bernard, and Dozier 2010; Vermeer et al. 2010). Indeed, the transition to childcare might elicit especially high cortisol levels (Ahnert et al. 2004; Bernard et al. 2015).

Extensive research documented the harmful effects of negative stress, such as neglect, maltreatment or other trauma, to brain development (Center on the Developing Child 2007, 2017). A prolonged elevation of cortisol could have negative effects, such as a weakened immune system (Watamura et al. 2010), inhibited cognitive development (Phillips, Fox, and Gunnar 2011) and greater sensitivity to stress in later life (Loman and Gunnar 2010). Cortisol elevations registered in childcare are not exceedingly high. At the same time, we do not know enough about this comparably milder form of stress and its effects on children's development (Gunnar and Herrera 2013; Vermeer and van Ijzendoorn 2006). Some elevation of cortisol seems to be necessary to reach the next steps of development (Center on the Developing Child 2017). According to Suhonen et al. (2018), learning might involve a balanced stress reaction, comprised by stress activation which alerts physical systems followed by stress regulation which enables the organism to

give an appropriate answer to a stressor, thereby avoiding a flight, fight or freeze response. Slightly heightened cortisol levels over brief periods of time might therefore be a sign of a learning process. Similarly, the Center on the Developing Child (2017) describes short and mild elevations of stress hormones in the context of supportive relationships as 'positive stress'. Positive stress is argued to be important for children's development and the establishment of a healthy stress response system.

Cortisol during the transition to childcare

To the best of our knowledge, only two studies have investigated toddlers' cortisol levels under the transition to childcare. Ahnert et al. (2004) measured morning cortisol during transition, upon arrival to childcare and 30 and 60 min later. In addition, they measured the same time points on days at home to assess the baseline of cortisol. On days when mothers were still present in the center, children showed just a slight cortisol elevation compared with at home. Yet this elevation was much higher for children with an insecure attachment style. Children exhibited the most elevated levels the first mornings separated from their mothers. At five months after entering childcare, levels were still higher compared with baseline but were significantly lower than during separation. Bernard et al. (2015) measured the cortisol levels of infants, preschoolers, and elementary school children during the first 10 weeks in a new childcare setting. Cortisol was measured in the morning and the afternoon on two days at home, on the first day in the new childcare setting, and on one day in the second, fourth, sixth, eighth, and tenth weeks. They observed decreasing cortisol between morning and afternoon at home and raising cortisol when in childcare. Age had a significant effect, and preschoolers (aged 18–60 months) showed more cortisol elevation than infants or elementary school children. Cortisol elevations continued to increase for all children during the first 10 weeks in the novel childcare setting.

To the best of our knowledge, no one has yet examined evening home levels in toddlers during their transition to childcare. Sumner, Bernard, and Dozier (2010), as well as Groeneveld et al. (2010), registered a decrease of cortisol in the evening after childcare, but the participants of those studies were not in the process of transitioning to childcare.

Factors related to cortisol elevation

Because of the lack of research, it is not clear how demographic child and childcare features relate to toddlers' cortisol levels during the transition to childcare and whether some groups are more influenced than others. Some studies that were conducted not during a transition but instead on regular childcare days, have found being male (Groeneveld et al. 2010; Ouellet-Morin et al. 2010) and of younger age (Ouellet-Morin et al. 2010) were associated with higher levels of cortisol. Legendre (2003) linked structural elements of childcare, such as a large group size, an unexpected high number of caregivers, small play space, and a great age difference between children in the group, to elevated cortisol levels in toddlers. Yet the results are not conclusive, and there are also non-findings regarding toddler's age (Groeneveld et al. 2010), gender (Drugli et al. 2018; Vermeer et al. 2010), as well as for elements of structural quality (Vermeer et al. 2010). To the best of our knowledge, nobody has yet explored whether toddlers with

siblings cope differently with transitions to childcare. Danish researchers reported qualitative experiences of toddlers adjusting easier to childcare when their older siblings attended the same center (Jensen et al. 2015).

The present study

The present study is part of a large Norwegian study called Thrive by Three, which investigates childcare provision for the youngest children. In Norway, the majority of one-year-old (74.8%) and two-year-old (93.6%) children attend childcare. Most of them spend up to or more than 41 h in childcare each week (Statistics Norway 2020). When children start to attend childcare for the first time, they are accompanied by a parent on the first days in the center. A recent survey answered by approximately half of all Norwegian childcare centers reported that most children are attended by one parent for three days. Yet there was a variation in practice, and many centers offered a week or flexible parental attendance. Most centers scheduled meetings and center visitations in order to get to know parents before the onset of childcare (Drugli, Buøen, and Grip 2017). All Norwegian childcare centers have to adhere to the national Framework Plan for Kindergartens (Norwegian Directorate for Education and Training 2017). Yet, childcare practice has been observed to differ greatly (Lekhal and Nasjonalt Folkehelseinstitutt 2013). A recent, large scale quality measurement by Bjørnestad and Os (2018) found Norwegian toddler childcare to be of middle-range quality.

Aims of the study

To adjust the practice of the transition to childcare better to toddlers needs, we need to identify the demanding stages of a transition and groups of children who might be challenged more than others. We will address the following research questions:

(1) How do cortisol levels change during the day (morning, afternoon, and evening) and during different phases of transition (together with parents, separated from parents, and four to six weeks in childcare)?
(2) How are gender, age at childcare entry, number of siblings, and group size related to cortisol levels during transition?

Based on the studies by Ahnert et al. (2004) and Bernard et al. (2015), we hypothesize that afternoon levels should be less elevated on the initial days in childcare accompanied by parents and then distinctly elevated in the afternoons compared with the mornings on the first days separated from parents and after four to six weeks in childcare. We hypothesize furthermore that cortisol levels will drop in the evening after children are picked up from childcare, as observed by Sumner, Bernard, and Dozier (2010) and Groeneveld et al. (2010). In addition, we want to study some basic child and childcare center variables and their influence on cortisol levels. We hypothesize that boys (Groeneveld et al. 2010; Ouellet-Morin et al. 2010), younger children (Ouellet-Morin et al. 2010), children without siblings (Jensen et al. 2015), and children in groups with a larger number of peers (Legendre 2003) might experience the transition as more challenging, therefore expressing higher levels of cortisol, particularly in the separation and follow-up phase.

Method

Participants

The present study was conducted on a subsample of the large Norwegian childcare study Thrive by Three. In 2017, leaders of childcare centers in seven municipalities in Eastern and Central Norway could enroll their center into the study. Thrive by Three applied a randomized-controlled design with an intervention group receiving a quality enhancement program and a control group. The sample of the present study consists of children from both groups. The quality intervention had not started when saliva sampling took place and, therefore, is unlikely to influence the present data.

Four to five of the Thrive by Three centers from each municipality were sampled. All children with valid consent from parents and who were starting childcare in autumn 2018 were eligible. Three centers were excluded from the present study because of a lack of eligible children. For details of recruitment, see Figures 1 and 2. For demographic information about the parents and children, see Tables 1 and 2.

Saliva sampling

In August 2018, we collected saliva samples from the children during their first weeks in childcare. Sampling occurred on six days in three phases: the second and third day accompanied by parents (acclimatization phase), the first and second day without parents (separation phase) and on two sequential days after four to six weeks in childcare (follow-up phase). Saliva sampling took place at 10 am and 3 pm in childcare and 6 pm at home.

Childcare centers were mailed saliva sampling kits with test tubes, swabs and written sampling guidance. Parents received a similar kit for their child from the childcare staff. Saliva samples were then collected at the according time points by employees in childcare and by parents at home. The Salimetrics' SalivaBio Children's Swab was used, which is intended for saliva sampling and validated for analysis of cortisol (Salimetrics 2019). Sampling was conducted in a playful manner to ease the procedure for the children.

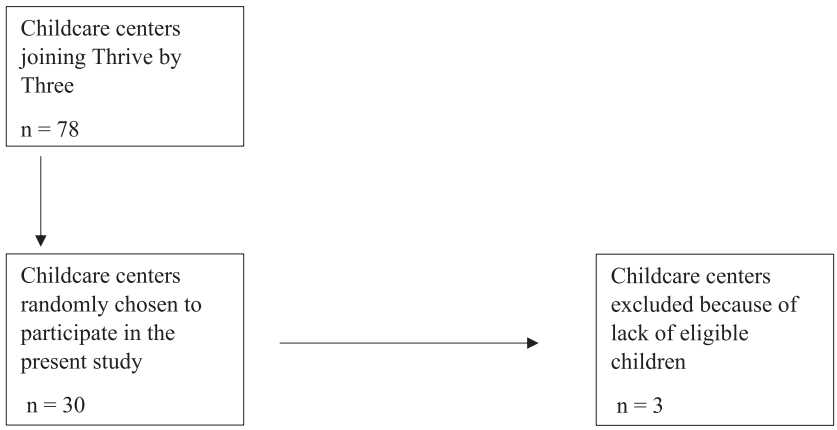


Figure 1. Flow chart on childcare center recruitment.

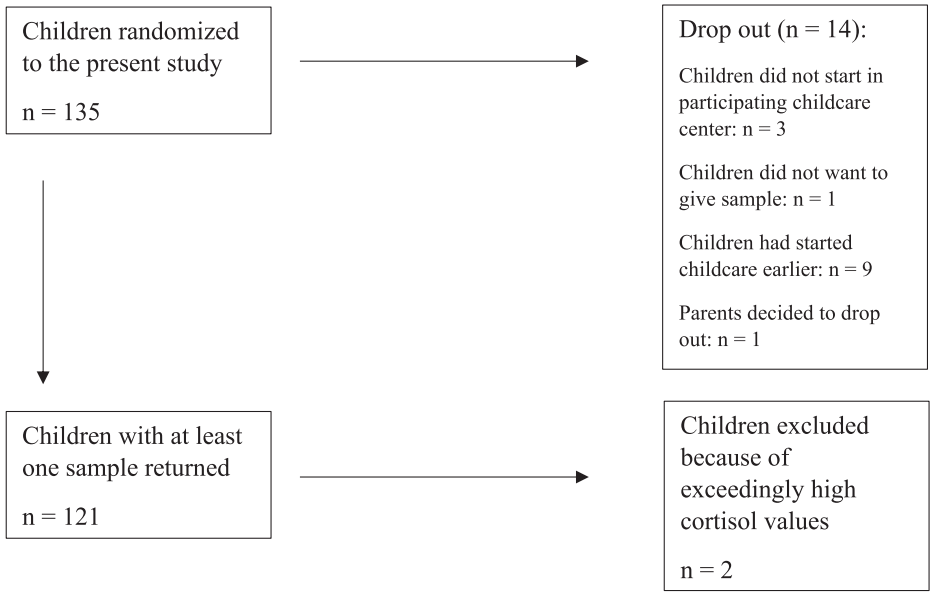


Figure 2. Flow chart on participant recruitment.

No stimulant was used. Childcare centers stored samples in household freezers until they were collected by the first author. The cortisol laboratory at the University of Trier, Germany, analyzed the saliva samples for their cortisol value by using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA) (Dressendörfer et al. 1992). The samples were destroyed after analysis.

We received 63.5% ($n = 1542$) of all possible samples. Among these, the laboratory excluded 7.1% ($n = 109$) of the samples because they contained too little saliva for analysis. After analysis, 3.4% ($n = 56$) of the values were excluded because of an unlogic high cortisol value. Based on recommendations from the laboratory in Trier and the

Table 1. Parent demography, mother ($n = 98$, missing = 22) and father ($n = 77$, missing = 36).

	% Mother	% Father
Language background		
Norwegian	93.9	90.9
Western	0	2.6
Non-Western	6.1	6.5
Marital status		
In a relationship	91.8	96.1
Single	8.2	3.9
Education		
Elementary school (9–10 years)	1.0	3.9
Finished secondary school	27.6	36.4
Up to 4 years with university education	33.7	22.1
More than 4 years of university education	37.8	37.7
Annual household income before tax		
Under 200,000 kroner	2.0	1.3
200,000–599,000 kroner	13.3	13.0
600,000–999,000 kroner	44.9	42.9
More than 1,000,000 kroner	40.8	42.9

Table 2. Child demography and group size.

	%/Mean	SD	Min	Max	*n*	*n* missing
Gender					119	0
Male	38.7%				46	
Female	61.3%				73	
Age at starting childcare in months	14.1	2.3	10	20	107	12
Number of siblings	0.8	0.998	0	8	111	8
Number of children in childcare group	11.76	2.006	8	16	49	0

laboratory at St. Olav's hospital in Trondheim, we decided to exclude values of more than 30 nmol/l because they likely reflected sickness or contamination. Over the course of this, we excluded two children from the analysis because of a constant pattern of exceedingly high values (Nicolson 2008). In total (1377 = 56.7%), the cortisol values from 119 children were available for analysis. Most children ($n = 115$) had one or more values missing, while four children had a complete set.

Questionnaire data

Parents answered an electronic questionnaire regarding their education, income, familial status, language background, and their child's gender, age at childcare entry, and number of siblings. Childcare staff gave electronic information on the number of children in the childcare groups.

Statistics

We used a linear mixed model with the base 10 logarithm of cortisol concentration in nmol/l as the dependent variable and individual as random effect. Phase and day within phase were included as a five-category covariate: the acclimatization day 1 (A1), acclimatization day 2 (A2), separation day 1 (S1), separation day 2 (S2), and follow-up (days 1 and 2) (F). The two sampling days of the follow-up phase were coded as the same day, because there was no difference between them. Time of day was included as a three-category covariate: morning, afternoon, and evening, and we included the interaction between day and time of day. Next, we analyzed the associations among gender, age in months at childcare entry, number of siblings, and number of children in the group unit. The continuous variables age and group size were dichotomized at their median into approximately equally sized groups: Age: up to 13 months versus 14 months and older; group size: up to 11 children versus 12 children and more. Sibling number was dichotomized into either having siblings or not having siblings. These variables were entered one at a time in the linear mixed model, including their two- and three-way interactions with day and time of day. We did not impute the missing values. The linear mixed model includes every data point of cortisol in the analysis, regardless of the number of missing data points from the same individual (Krueger and Tian 2004). Normality of residuals was checked by visual inspection of QQ plots. Cortisol levels above 30 nmol/l were excluded from the analysis and regarded as missing values. We regard two-sided p-values <0.05 as statistically significant and report 95% confidence intervals (CI) where relevant. Analyses were carried out in SPSS26.

Ethics

Participation was voluntary for municipalities, childcare centers, caregivers, and parents. Both parents gave electronic consent for their child's participation. Caregivers gave electronic consent as well. Withdrawal of consent was possible at all stages of the study.

We did not sample saliva from children who refused to participate in the sampling procedure.

Thrive by Three, and hence the present study, are approved by the Regional Committees for Medical and Health Research Ethics and the Norwegian Center for Research Data.

Results

First, we compared cortisol levels both between days during the different transition phases and times of day. Table 3 and Figure 3 show the number of observations and estimated marginal means for each day and time of day.

Changes throughout the days

As seen in Figure 3, there was a slight increase in morning cortisol level over the days of the transition. The *p*-values comparing each day with day A1 are shown in Figure 4. Changes in morning cortisol were not statistically significant. For the afternoon, there was a higher increase in cortisol levels over the phases. As seen in Figure 4, this was particularly the case for S2 compared with A1 (p=0.009). For the evening, there was a slight decrease in cortisol (Figure 4). This decrease was statistically significant when comparing A1 and F.

Differences throughout time of day

On all days, the afternoon cortisol levels were higher than the morning cortisol levels. These differences were highest and statistically significant on day S1, S2 and F (see Figure 5). Untransformed average cortisol increased 42.5% from morning to afternoon on day S2. Respectively, it increased 31.5% on day S1 and 12% on day F. The evening

Table 3. Estimated marginal means of log10-transformed cortisol (nmol/l).

Day	Time	n	Mean	95% confidence interval		
A1	Morning	78	0.592	0.515	to	0.669
	Afternoon	40	0.622	0.518	to	0.727
	Evening	93	0.262	0.191	to	0.334
A2	Morning	83	0.605	0.530	to	0.680
	Afternoon	51	0.691	0.597	to	0.784
	Evening	89	0.193	0.120	to	0.265
S1	Morning	83	0.632	0.557	to	0.707
	Afternoon	66	0.745	0.662	to	0.828
	Evening	80	0.215	0.139	to	0.292
S2	Morning	77	0.664	0.587	to	0.742
	Afternoon	58	0.793	0.705	to	0.881
	Evening	80	0.174	0.098	to	0.251
F	Morning	175	0.671	0.614	to	0.727
	Afternoon	162	0.740	0.682	to	0.799
	Evening	162	0.140	0.082	to	0.198

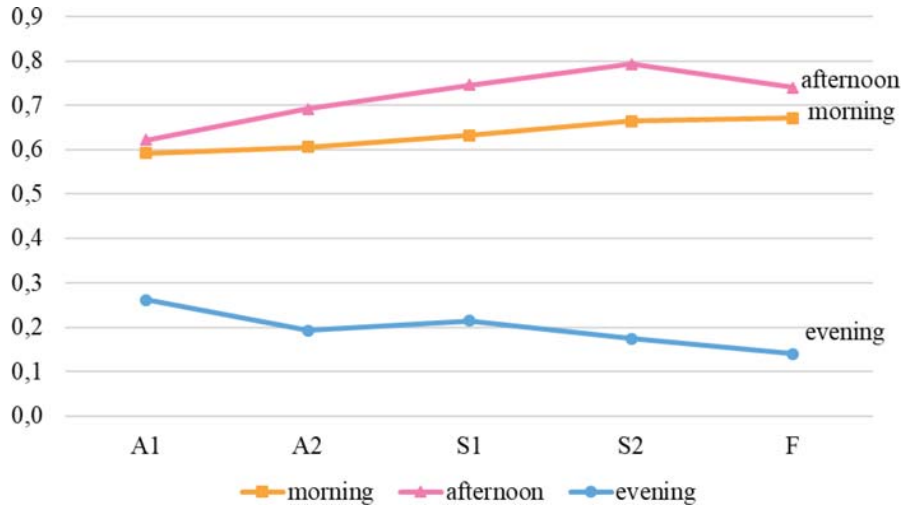


Figure 3. Estimated marginal means of cortisol in nmol/l (log10-transformed).

levels were substantially lower than the morning and afternoon levels on all days. These differences were statistically highly significant. As can be seen in Table 4, the cortisol levels showed a greater standard deviation on the afternoon of S2 compared with other afternoons or the corresponding evenings.

Influence of age, gender, number of siblings and group size on cortisol

Second, we investigated how age at childcare entry, gender, group size, and number of siblings influenced cortisol activity. Gender and group size did not yield statistically

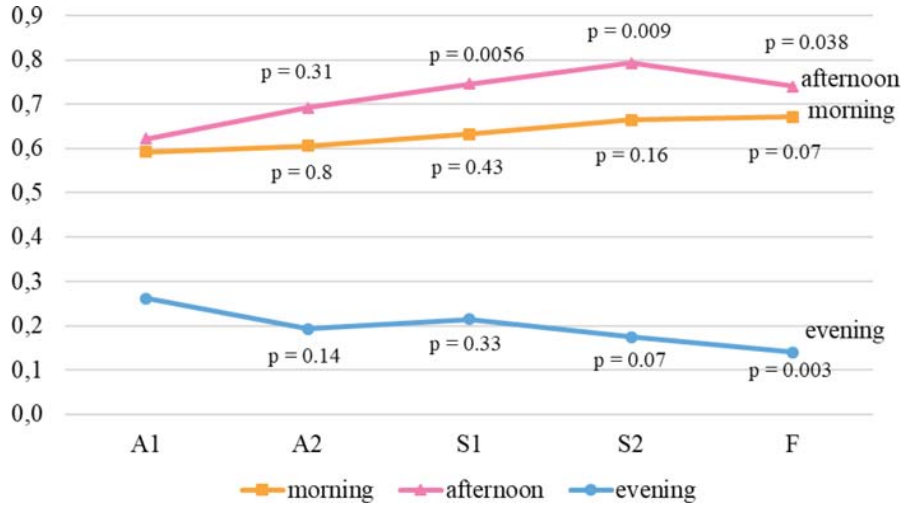


Figure 4. Estimated marginal means of cortisol in nmol/l (log10-transformed) with *p*-values of change compared to A1.

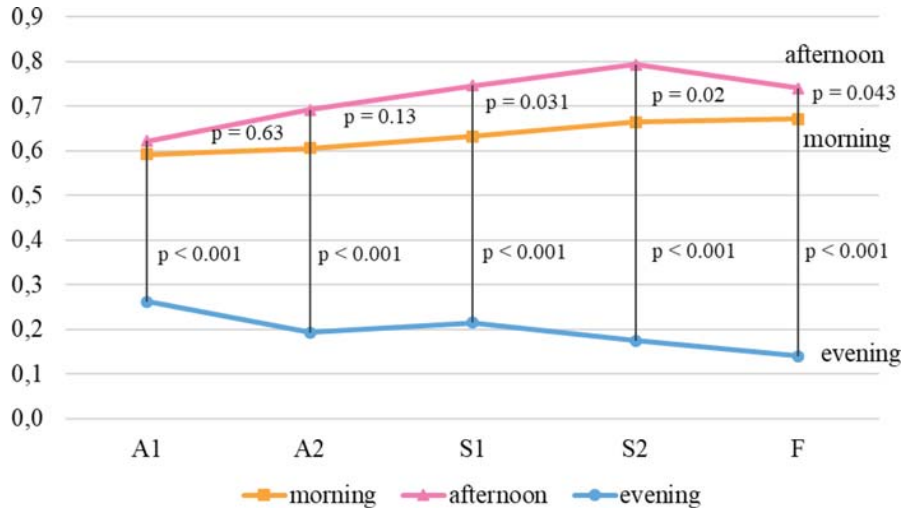


Figure 5. Estimated marginal means of cortisol in nmol/l (log10-transformed) with *p*-values of change compared to morning.

relevant effects. Children with siblings showed higher morning cortisol levels than children without siblings, but the effects were not significant. The corresponding graphs are shown in the appendix (Figures A1–A6). Age produced a highly significant main effect. Also, the interaction between time of day and age was significant. Table 5 and Figures 6 and 7 show the estimated marginal means for the respective age groups.

Table 6 illustrates the difference between the age groups by subtracting the estimated marginal mean of the older group from the estimated marginal mean of the younger group. Morning cortisol was higher for younger children on A1 and A2. Differences in morning cortisol were minor on other days. Afternoon cortisol was higher for younger children on A1 and F and slightly lower on A2. The difference was minor on the separation days. Younger children's evening levels were well above the levels of the older group on all days of the transition, with an especially large difference on the evening of day S2.

Table 4. Descriptive statistics of untransformed cortisol (nmol/l).

Day	Time	*n*	Mean	SD	Minimum	Maximum
A1	Morning	78	5.12	4.73	0.68	29.30
	Afternoon	40	5.38	4.05	0.71	19.68
	Evening	93	2.75	3.37	0.22	25.03
A2	Morning	83	5.31	4.49	0.82	29.87
	Afternoon	51	6.72	5.32	0.84	23.76
	Evening	89	2.44	3.20	0.10	25.99
S1	Morning	83	5.39	4.22	1.40	24.28
	Afternoon	66	7.09	5.14	1.24	24.36
	Evening	80	2.65	3.18	0.20	14.55
S2	Morning	77	5.77	4.41	0.77	22.80
	Afternoon	58	8.22	6.70	1.29	29.85
	Evening	80	2.32	2.49	0.10	12.18
F	Morning	175	5.83	4.43	0.92	23.88
	Afternoon	162	6.53	4.51	1.03	24.18
	Evening	162	2.43	3.71	0.01	25.45
Valid *N* (listwise)		4				

Table 5. Estimated marginal means of log10-transformed cortisol (nmol/l) by age.

Day	Time	Age in months	*n*	Mean	95% confidence interval		
A1	Morning	≤13	31	0.658	0.539	to	0.778
		>13	35	0.522	0.409	to	0.634
	Afternoon	≤13	16	0.675	0.512	to	0.839
		>13	16	0.526	0.364	to	0.689
	Evening	≤13	38	0.385	0.276	to	0.494
		>13	44	0.147	0.045	to	0.248
A2	Morning	≤13	36	0.661	0.549	to	0.772
		>13	37	0.546	0.436	to	0.656
	Afternoon	≤13	24	0.663	0.528	to	0.797
		>13	20	0.699	0.553	to	0.846
	Evening	≤13	25	0.244	0.131	to	0.358
		>13	43	0.117	0.014	to	0.219
S1	Morning	≤13	29	0.593	0.469	to	0.716
		>13	43	0.631	0.528	to	0.733
	Afternoon	≤13	30	0.748	0.626	to	0.869
		>13	28	0.677	0.552	to	0.802
	Evening	≤13	30	0.273	0.152	to	0.395
		>13	40	0.104	−0.002	to	0.210
S2	Morning	≤13	30	0.680	0.558	to	0.801
		>13	37	0.619	0.509	to	0.729
	Afternoon	≤13	26	0.806	0.676	to	0.936
		>13	25	0.783	0.651	to	0.915
	Evening	≤13	33	0.349	0.232	to	0.465
		>13	37	−0.028	−0.138	to	0.082
F	Morning	≤13	76	0.680	0.598	to	0.763
		>13	87	0.639	0.562	to	0.716
	Afternoon	≤13	67	0.778	0.691	to	0.865
		>13	85	0.674	0.596	to	0.752
	Evening	≤13	66	0.225	0.137	to	0.312
		>13	84	0.032	−0.046	to	0.110

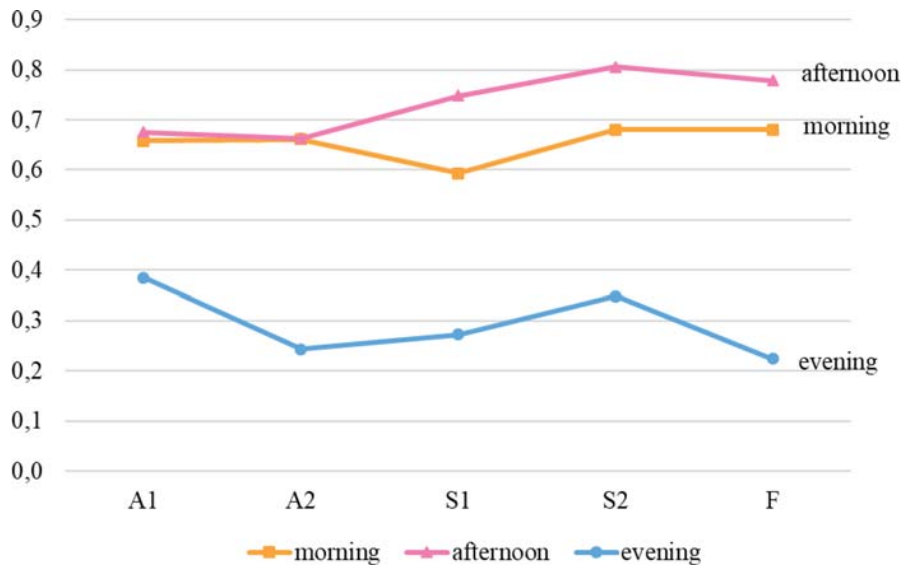


Figure 6. Estimated marginal means of cortisol in nmol/l (log10-transformed) for children up to 13 months.

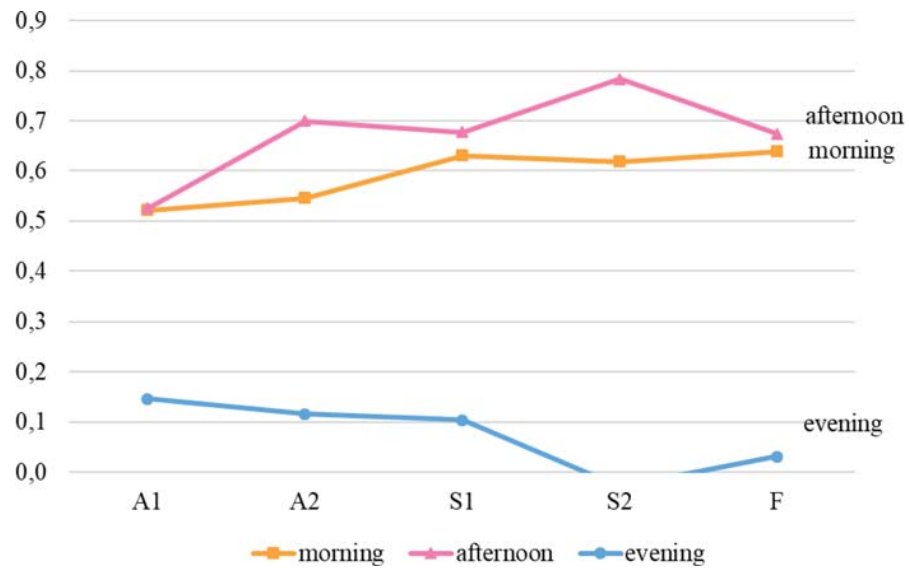


Figure 7. Estimated marginal means of cortisol in nmol/l (log10-transformed) for children 14 months and older.

Table 6. Differences of estimated marginal means by age (≤13–>13).

Day	Time	Difference
A1	Morning	0.136
	Afternoon	0.149
	Evening	0.238
A2	Morning	0.115
	Afternoon	−0.036
	Evening	0.127
S1	Morning	−0.038
	Afternoon	0.71
	Evening	0.169
S2	Morning	0.061
	Afternoon	0.023
	Evening	0.377
F	Morning	0.041
	Afternoon	0.104
	Evening	0.193

Discussion

In the present study, we investigated toddlers' cortisol activity during their transition to childcare. We compared cortisol levels under acclimatization with parents, separation from parents and after four to six weeks in childcare in the morning and afternoon in childcare, as well as in the evening at home. We found significantly elevated cortisol levels on the afternoons of separation and follow-up days. However, cortisol levels had distinctly dropped on all evenings. Additionally, we tested the effect of the variables age, gender, number of siblings, and group size on cortisol activity. Age had a statistically significant effect on cortisol levels.

Primary findings: the cortisol curve

Afternoon cortisol levels were only slightly elevated on days together with parents in childcare. A nearly similar pattern was observed by Ahnert et al. (2004). This suggests that the presence of a parent may have buffered the stress induced by the new setting, at least to some degree (Gunnar and Donzella 2002). Children might have a greater capacity to explore their new surroundings and interact with caregivers in the presence of a parent who can help regulate their emotions (Schore and Schore 2008) and bridge the gap between home and the new childcare context (Bronfenbrenner 1979). Therefore, extending the number of days together with parents if necessary may facilitate children's familiarization with the center and caregivers (Brooker 2008). Ahnert et al. (2004) did not find an effect of the number of days mothers spent in childcare on cortisol elevation during separation. Qualitative findings indicate that familiarity with the new context, especially with caregivers, preferably with a primary key person among them, helped children to settle in better (Brooker 2008; Ebbeck and Yim 2009).

Children showed distinctly elevated cortisol levels on the afternoons of separation. Indeed, the absence of parents seems to elicit distress, especially on the second afternoon. Ahnert et al. (2004) also reported a clear stress response to separation from mothers. Toddlers might have diminished capacity to handle distress on the second afternoon alone, or they might have realized that parents are not coming back soon (Ahnert et al. 2004; Ahnert and Lamb 2003). A greater variation in the afternoon cortisol levels of the second day alone than on the other days in childcare might be an indication that some children experience more stress during their separation phase. The absence of parents might challenge children because of a diminished support to regulate feelings (Schore and Schore 2008). Having known children only for a few days, caregivers might not be able yet to soothe them well (Elfer, Goldschmied, and Selleck 2012). In addition, some children might experience little control over when and if parents are coming back, because of earlier experience (O'Connor 2013) or because they have not developed object permanence yet, meaning they do not know that parents exist when they are not there (Shaffer and Kipp 2014). Because the initial days of separation seem to be the most demanding part of transition for children, reduced hours might help to keep stress activation in balance (Vermeer and Groeneveld 2017). During our data collection, it was a frequent, verbally given feedback from the childcare centers that the children spent very short days together with their parents in childcare, whereas the separation days were comparably longer. By then, most parents had started working full time again after parental leave.

Afternoon cortisol levels were still somewhat elevated after four to six weeks in childcare. Children might have still been in the process of adapting to childcare. These elevated levels might also indicate that children experience childcare as challenging. This corresponds to the findings of the studies by both Bernard et al. (2015) and Ahnert et al. (2004), who reported elevated cortisol at a follow-up measurement after 10 weeks and five months. To help children to keep their stress activation balanced, it might be beneficial that parents continue with short days after the initial transition (Vermeer and Groeneveld 2017) and stay present with children for more days if necessary and possible (Brooker 2008). Toddlers who spend long hours in childcare have been found to show more cortisol elevation (Drugli et al. 2018). Although some parents benefit

from flexible working hours in Norway, others need to adhere to a commute or to fixed schedules, hence depending on full-time childcare soon after the transition. Flexible parent leaves during the first weeks after starting childcare could help parents adjust the transitions to their child's needs. Norway already has generous parent provisions, yet society still might profit from better transitions because this could potentially lead to less strain on children and thereby fewer days parents would have to stay home with sick children (Watamura et al. 2010). Process quality is likely an important factor when it comes to regulating stress in toddlers' everyday childcare experience. Studies including older children have observed a connection between relational quality and cortisol levels in childcare (Badanes, Dmitrieva, and Watamura 2012; Sajaniemi et al. 2011). For toddlers, some studies have found a relation between elements of process quality and HPA activity (Groeneveld et al. 2010; Vermeer et al. 2010), while others did not (Drugli et al. 2018). The matter needs to be researched further (Vermeer and van Ijzendoorn 2006).

Evening cortisol levels were markedly lower than afternoon levels on all days. It seems that children experienced relief when they were at home after childcare. To the best of our knowledge, our study is the first to investigate cortisol levels in the evening after childcare during transition, thereby providing new knowledge. The findings correspond to the results of Groeneveld et al. (2010) and Sumner, Bernard, and Dozier (2010), who also registered a drop in evening cortisol. Those studies were conducted on regular childcare days, though, not during a transition.

Secondary findings: age

Children showed different patterns of cortisol activity according to age. These findings must be interpreted with caution, as children's stress regulation may still be under development. A study by Watamura et al. (2004) indicates that maturation of the HPA axis continues through the first three years of life. Cortisol levels of approximately 3-year-old children were overall lower than those of 1- and 2-year-old children. Blair et al. (2011) suggest as well that baseline cortisol levels gradually sink throughout toddlerhood. Out of practical reasons we were not able to assess children's cortisol baseline at home before starting childcare. This might have enhanced our understanding of the effect of age on cortisol.

We compared two age groups: up to 13 months and from 14 months up. Older children showed slightly higher afternoon cortisol levels on the third day after starting childcare while parents were still present in childcare. Parents might have left older children sooner alone, or they might not have kept as close to them as the parents of younger children had. There is reason to believe that separation occurs already on the third day of childcare in the Norwegian context (Drugli, Buøen, and Grip 2017). Older toddlers might have also been urged into more peer play by parents and caregivers. Contact with peers is suspected to cause an increase in cortisol (Gunnar and Quevedo 2007).

Younger children have been found to have a higher baseline of cortisol by Blair et al. (2011), yet when comparing afternoon levels throughout the phases, we suspect that there might be an activation of younger children in the follow-up phase. While the afternoon level of older children decreased markedly between the separation and follow-up phase, younger children were equally activated on the second afternoon of separation

and the follow-up afternoons. Children younger than 14 months may need more time to adjust to childcare. They might be emotionally less mature and might have a lower capacity to regulate the difficult feelings accompanying the transition (Schore and Schore 2008). Furthermore, younger children might have less social competence and, therefore, might experience peer contact and play as more demanding (Denham et al. 2003). Caregivers have to facilitate interaction between peers (Brooker 2008) and be attentive toward new children also after the initial transition to notice the, at times, subtle signs of discomfort and struggle (Datler et al. 2012). Adjustment to childcare is an extended process that might vary in length for different children (Brooker 2008; Datler et al. 2012). This is also highlighted by the concept of Inserimento in Reggio Emilia childcare (Degotardi and Pearson 2014). It might be beneficial that childcare centers focus on familiarizing children with routines (Brooker 2008) and reduce stimuli and activities in the afternoon, as children have been observed to be tired then (Undheim and Drugli 2012). Indeed, afternoon levels did not differ much by age during separation, suggesting that the absence of parents is relatively challenging, regardless of age.

Toddlers showed different evening levels according to age. Children younger than 14 months had higher evening cortisol levels than older children on all days. Younger children might have a flatter decline of cortisol due to maturation of the HPA axis (Blair et al. 2011; Watamura et al. 2004). It is also possible that younger children did not unwind as much and were more tense than older children at home after childcare. Yet also for younger children, cortisol levels dropped from afternoon to evening. Although there was no indication of an extended stress response, the matter should be researched more thoroughly. A prolonged activation of HPA has been linked to unfavorable outcomes, such as an inhibited immune system (Watamura et al. 2010), greater risk for a future vulnerability for stress (Loman and Gunnar 2010), and an inhibition of cognitive development (Phillips, Fox, and Gunnar 2011).

It is also possible that some younger children have a nap after childcare, resulting in an elevation of cortisol, which is called a cortisol awakening response (CAR) (Tervahartiala et al. 2019). However, evening napping has been observed to not produce much cortisol (Tribble et al. 2015). To alleviate tension, children might benefit from calm evenings with focused care from parents (Ahnert and Lamb 2003). The distinct decline of evening cortisol indicates that most parents soothed children after coming home. Ahnert, Rickert, and Lamb (2000) reported that mothers gave more attention in the evening to toddlers attending childcare compared with mothers whose children did not attend childcare. This has been observed to be a powerful regulator of the HPA axis (Gunnar and Donzella 2002).

Strengths and limitations

The present study has some noteworthy strengths. We enrolled a larger number of participants than other cortisol studies on toddlers during transition to childcare (Ahnert et al. 2004; Bernard et al. 2015). Two days of measurement in three phases gave a good overview over the developments during the different stages of transition to childcare. Cortisol was measured in a full-day circle under transition for the first time.

The findings of the present study also need to be understood in the light of certain limitations. We have not assessed cortisol levels at home before starting childcare. Therefore, acclimatization days were applied as a baseline. Previous research gives strong indications that home cortisol is declining and that afternoon levels are lower compared with cortisol in childcare (Ahnert et al. 2004; Bernard et al. 2015; Drugli et al. 2018; Vermeer and van Ijzendoorn 2006). We assume this to be the case for our participants. A further study should assess this baseline level and investigate it for age differences.

There is a high percentage of missing cortisol data on certain time points, especially on afternoons together with parents in childcare. The linear mixed model analysis can process missing data better than other methods of analysis (Krueger and Tian 2004).

We have not controlled for napping, so a cortisol awakening response might be partly responsible for afternoon elevations (Tervahartiala et al. 2019; Tribble et al. 2015). Yet because napping should approximately have the same effect each afternoon, we might be able to neglect the difference. Furthermore, we have not controlled for the waking-up time in the morning and children's morning cortisol might still be elevated by the morning CAR (Bäumler et al. 2013).

Cortisol levels are not assessed beyond four to six weeks after starting in childcare. Participants with a non-Norwegian language background are underrepresented in our sample (Statistics Norway 2019c). Parental education is well above the Norwegian average (Statistics Norway 2019a), and their income is slightly above regional average for families with young children (Statistics Norway 2019b). Children from a background with higher familial risk might show different cortisol patterns in the context of childcare (Berry et al. 2014).

Implications and future research

The findings of the present study hold implications for parents, caregivers, and policy-makers. More time together with parents in childcare and shorter days during the separation phase could facilitate children's transition. Parents should provide soothing and attention for their children in the evening after a day in childcare. Caregivers need to be attentive during children's first months in childcare and focus especially on silent cues of discomfort. It is advisable that childcare centers provide calm afternoons and prioritize children's familiarization with the caregivers, surroundings, and routines. Caregivers should also facilitate peer play. Flexible leave possibilities for parents during the children's first weeks in childcare could help to adjust the transition practice to the needs of individual children.

There is little systematic research on toddlers' transitions into childcare. Future studies should try to identify children who are more exposed to cortisol elevation during this transition than others. A further study should incorporate a larger sample of children to find the factors linked to greater elevations of cortisol during the transition to childcare.

Process quality in childcare (Vermeer and van Ijzendoorn 2006), as well as, children's temperament (Dettling et al. 2000) and attachment style (Ahnert et al. 2004) might have an influence and need to be explored further. Also, the influence of transition practices on cortisol elevations, such as the number of days parents spend in childcare, primary contact approach, and cooperation between childcare and home should be investigated.

Further studies should also explore the effect of age at childcare entry on cortisol levels a couple of months after transition.

Acknowledgements

The authors thank all children, parents and caregivers for participating in this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Thrive by Three and the present study were funded by The Research Council of Norway under the program 'BEDREHELSE'.

ORCID

K. Nystad http://orcid.org/0000-0003-0844-3642
M. B. Drugli http://orcid.org/0000-0002-2325-8552
S. Lydersen http://orcid.org/0000-0001-6613-8596
R. Lekhal http://orcid.org/0000-0002-3750-4275
E. S. Buøen http://orcid.org/0000-0001-7120-4586

References

Ahnert, L., M. R. Gunnar, M. E. Lamb, and M. Barthel. 2004. "Transition to Child Care: Associations With Infant-Mother Attachment, Infant Negative Emotion, and Cortisol Elevations." *Child Development* 75 (3): 639–650. doi:10.1111/j.1467-8624.2004.00698.x.

Ahnert, L., and M. E. Lamb. 2003. "Shared Care: Establishing a Balance Between Home and Child Care Settings." *Child Development* 74 (4): 1044–1049. doi:10.1111/1467-8624.00587.

Ahnert, L., H. Rickert, and M. E. Lamb. 2000. "Shared Caregiving: Comparisons Between Home and Child-care Settings." *Developmental Psychology* 36 (3): 339–351. doi:10.1037/0012-1649.36.3.339.

Badanes, L. S., J. Dmitrieva, and S. E. Watamura. 2012. "Understanding Cortisol Reactivity Across the Day at Child Care: The Potential Buffering Role of Secure Attachments to Caregivers." *Early Childhood Research Quarterly* 27 (1): 156–165. doi:10.1016/j.ecresq.2011.05.005.

Bäumler, D., C. Kirschbaum, M. Kliegel, N. Alexander, and T. Stalder. 2013. "The Cortisol Awakening Response in Toddlers and Young Children." *Psychoneuroendocrinology* 38 (11): 2485–2492. doi:10.1016/j.psyneuen.2013.05.008.

Belsky, J. 2001. "Emanuel Miller Lecture Developmental Risks (Still) Associated with Early Child Care." *Journal of Child Psychology and Psychiatry* 42 (7): 845–859. doi:10.1111/1469-7610.00782.

Bernard, K., E. Peloso, J.P. Laurenceau, Z. Zhang, and M. Dozier. 2015. "Examining Change in Cortisol Patterns During the 10-week Transition to a New Child-care Setting." *Child Development* 86 (2): 456–471. doi:10.1111/cdev.12304.

Berry, D., C. Blair, A. Ursache, M. Willoughby, P. Garrett-Peters, L. Vernon-Feagans, M. Bratsch-Hines, W. R. Mills-Koonce, and D. A. Granger. 2014. "Child Care and Cortisol Across Early Childhood: Context Matters." *Developmental Psychology* 50 (2): 514–525. doi:10.1037/a0033379.

Bjørnestad, E., and E. Os. 2018. "Quality in Norwegian Childcare for Toddlers Using ITERS-R." *European Early Childhood Education Research Journal* 26 (1): 111–127. doi:10.1080/1350293X.2018.1412051.

Blair, C., C. C. Raver, D. Granger, R. Mills-Koonce, and L. Hibel. 2011. "Allostasis and Allostatic Load in the Context of Poverty in Early Childhood." *Development and Psychopathology* 23 (3): 845–857. doi:10.1017/s0954579411000344.

Bronfenbrenner, U. 1979. *The Ecology of Human Development*. London: Harvard University Press.

Brooker, L. 2008. *Supporting Transitions in the Early Years: Supporting Early Learning*. Maidenhead: McGraw-Hill Education.

Center on the Developing Child. 2007. "The Impact of Early Adversity on Child Development (InBrief)." Accessed August 10, 2020. https://developingchild.harvard.edu/wp-content/uploads/2015/05/inbrief-adversity-1.pdf

Center on the Developing Child. 2012. "Establishing a Level Foundation for Life: Mental Health Begins in Early Childhood: Working Paper No. 6." Accessed August 10, 2020. https://developingchild.harvard.edu/wp-content/uploads/2008/05/Establishing-a-Level-Foundation-for-Life-Mental-Health-Begins-in-Early-Childhood.pdf.

Center on the Developing Child. 2017. "Toxic Stress." Accessed June 7, 2018. https://developingchild.harvard.edu/science/key-concepts/toxic-stress/.

Cryer, D., L. Wagner-Moore, M. Burchinal, N. Yazejian, S. Hurwitz, and M. Wolery. 2005. "Effects of Transitions to New Child Care Classes on Infant/Toddler Distress and Behavior." *Early Childhood Research Quarterly* 20 (1): 37–56. doi:10.1016/j.ecresq.2005.01.005.

Datler, W., M. Datler, and A. Funder. 2010. "Struggling Against a Feeling of Becoming Lost: A Young Boy's Painful Transition to Day Care." *Infant Observation* 13 (1): 65–87. doi:10.1080/13698031003606659.

Datler, W., K. Ereky-Stevens, N. Hover-Reisner, and L. E. Malmberg. 2012. "Toddlers' Transition to Out-of-Home Day Care: Settling Into a New Care Environment." *Infant Behavior and Development* 35 (3): 439–451. doi:10.1016/j.infbeh.2012.02.007.

Degotardi, S., and E. Pearson. 2014. *Relationship Worlds of Infants and Toddlers: Multiple Perspectives from Early Years Theory and Practice*. Berkshire: Open Education Press. McGraw-Hill Education.

Denham, S. A., K. A. Blair, E. DeMulder, J. Levitas, K. Sawyer, S. Auerbach-Major, and P. Queenan. 2003. "Preschool Emotional Competence: Pathway to Social Competence?" *Child Development* 74 (1): 238–256. doi:10.1111/1467-8624.00533.

Dettling, A. C., S. W. Parker, S. Lane, A. Sebanc, and M. R. Gunnar. 2000. "Quality of Care and Temperament Determine Changes in Cortisol Concentrations Over the Day for Young Children in Childcare." *Psychoneuroendocrinology* 25 (8): 819–836. doi:10.1016/S0306-4530(00)00028-7.

Dressendörfer, R. A., C. Kirschbaum, W. Rohde, F. Stahl, and C. J. Strasburger. 1992. "Synthesis of a Cortisol-Biotin Conjugate and Evaluation as a Tracer in an Immunoassay for Salivary Cortisol Measurement." *The Journal of Steroid Biochemistry and Molecular Biology* 43 (7): 683–692. doi:10.1016/0960-0760(92)90294-S.

Drugli, M. B. 2017. "Foreldre bør være vaktbikkje når ettåringen skal begynne i barnehagen." Adresseavisen, August 7. Accessed April 20, 2020. https://www.midtnorskdebatt.no/meninger/2017/08/07/Foreldre-b%C3%B8r-v%C3%A6re-vaktbikkje-n%C3%A5r-ett%C3%A5ringen-skal-begynne-i-barnehagen-15111094.ece.

Drugli, M. B., E. S. Buøen, and M. S. Grip. 2017. "Rutiner for tilvenning i norske barnehager." *Barnehagefolk* 1: 92–98.

Drugli, M. B., E. Solheim, S. Lydersen, V. Moe, L. Smith, and T. S. Berg-Nielsen. 2018. "Elevated Cortisol Levels in Norwegian Toddlers in Childcare." *Early Child Development and Care*, 1–12. doi:10.1080/03004430.2016.1278368.

Ebbeck, M., and H. Y. B. Yim. 2009. "Rethinking Attachment: Fostering Positive Relationships Between Infants, Toddlers and Their Primary Caregivers." *Early Child Development and Care* 179 (7): 899–909. doi:10.1080/03004430701567934.

Elfer, P., E. Goldschmied, and D. Y. Selleck. 2012. *Key Persons in the Early Years: Building Relationships for Quality Provisions in Early Years Settings and Primary Schools*. London: Routledge.

Ereky-Stevens, K., A. Funder, T. Katschnig, L. E. Malmberg, and W. Datler. 2018. "Relationship Building Between Toddlers and New Caregivers in Out-of-Home Childcare: Attachment Security and Caregiver Sensitivity." *Early Childhood Research Quarterly* 42: 270–279. doi:10.1016/j.ecresq.2017.10.007.

Groeneveld, M. G., H. J. Vermeer, M. H. van IJzendoorn, and M. Linting. 2010. "Children's Wellbeing and Cortisol Levels in Home-based and Center-based Childcare." *Early Childhood Research Quarterly* 25 (4): 502–514. doi:10.1016/j.ecresq.2009.12.004.

Gunnar, M. R., and B. Donzella. 2002. "Social Regulation of the Cortisol Levels in Early Human Development." *Psychoneuroendocrinology* 27 (1): 199–220. doi:10.1016/S0306-4530(01)00045-2.

Gunnar, M. R., and A. M. Herrera. 2013. "The Development of Stress Reactivity: A Neurobiological Perspective." In *The Oxford Handbook of Developmental Psychology: 2: Self and Other: Vol.2*, edited by P. D. Zelazo, 45–80. Oxford: Oxford University Press.

Gunnar, M. R., and K. Quevedo. 2007. "The Neurobiology of Stress and Development." *Annual Review of Psychology* 58 (1): 145–173. doi:10.1146/annurev.psych.58.110405.085605.

Humberset, K., and E. R. Andersen. 2018. "Linn og Rebaz har satt av en uke til sønnens oppstart i barnehagen: – Foreldre bruker mer tid nå enn tidligere." Adresseavisen, August 9. Accessed March 10, 2020. https://www.adressa.no/familieogoppvekst/Linn-og-Rebaz-har-satt-av-en-uke-til-sonnens-oppstart-i-barnehagen–Foreldre-bruker-mer-tid-na-enn-tidligere-12216b.html.

Jensen, T.M., S. Broström, O. H. Hansen, L. Svinth, and A.S. Jensen. 2015. *Barnet i Centrum - Evaluering*. Copenhagen: Århus University.

Kirschbaum, C., and D. Hellhammer. 1994. "Salivary Cortisol in Psychoneuroendocrine Research: Recent Developments and Applications." *Psychoneuroendocrinology* 19: 313–333. doi:10.1016/0306-4530(94)90013-2.

Kirschbaum, C., R. Steyer, M. Eid, U. Patalla, P. Schwenkmezger, and D. H. Hellhammer. 1990. "Cortisol and Behavior: 2. Application of a Latent State-Trait Model to Salivary Cortisol." *Psychoneuroendocrinology* 15 (4): 297–307. doi:10.1016/0306-4530(90)90080-S.

Krueger, C., and L. Tian. 2004. "A Comparison of the General Linear Mixed Model and Repeated Measures ANOVA Using a Dataset with Multiple Missing Data Points." *Biological Research for Nursing* 6 (2): 151–157. doi:10.1177/1099800404267682.

Legendre, A. 2003. "Environmental Features Influencing Toddlers' Bioemotional Reactions in Day Care Centers." *Environment and Behavior* 35 (4): 523–549. doi:10.1177/0013916503035004005.

Lekhal, R. 2012. "Do Type of Childcare and Age of Entry Predict Behavior Problems During Early Childhood? Results from a Large Norwegian Longitudinal Study." *International Journal of Behavioral Development* 36 (3): 197–204. doi:10.1177/0165025411431409.

Lekhal, R., and Nasjonalt Folkehelseinstitutt. 2013. *Variasjon I Barnehagekvalitet: Den Norske Mor Og Barn-undersøkelsen: Beskrivelser Fra Første Datainnsamling Fra Barnehagene*. Oslo: Folkehelseinstituttet.

Loman, M. M., and M. R. Gunnar. 2010. "Early Experience and the Development of Stress Reactivity and Regulation in Children." *Neuroscience and Biobehavioral Reviews* 34 (6): 867–876. doi:10.1016/j.neubiorev.2009.05.007.

Markström, A., and M. Simonsson. 2017. "Introduction to Preschool: Strategies for Managing the Gap Between Home and Preschool." *Nordic Journal of Studies in Educational Policy* 3 (2): 179–188. doi:10.1080/20020317.2017.1337464.

NICHD – Early Child Care Research Network. 2002. "Child-care Structure → Process → Outcome: Direct and Indirect Effects of Child-care Quality on Young Children's Development." *Psychological Science* 13 (3): 199–206. doi: 10.1111/1467-9280.00438.

Nicolson, N. A. 2008. "Measurement of Cortisol." In *Handbook of Physiological Research Methods in Health Psychology*, edited by L. Luecken and L. Gallo, 37–74. Los Angeles: SAGE Publications.

Norwegian Directorate for Education and Training. 2017. "Framework Plan for Kindergartens: Contents and Tasks." Accessed August 10, 2020. https://www.udir.no/globalassets/filer/barnehage/rammeplan/framework-plan-for-kindergartens2-2017.pdf.

O'Connor, A. 2013. *Understanding Transitions in the Early Years: Supporting Change Through Attachment and Resilience*. London: Routledge.

Organisation for Economic Co-operation and Development. 2017. "Starting Strong 2017: Key OECD Indicators on Early Childhood Education and Care." Paris: OECD Publishing. Accessed April 21, 2020. https://read.oecd-ilibrary.org/education/starting-strong-2017_9789264276116-en.

Ouellet-Morin, I., R. E. Tremblay, M. Boivin, M. Meaney, M. Kramer, and S. M. Côté. 2010. "Diurnal Cortisol Secretion at Home and in Child Care: A Prospective Study of 2-Year-Old Toddlers." *Journal of Child Psychology and Psychiatry* 51 (3): 295–303. doi:10.1111/j.1469-7610.2009.02167.x.

Phillips, D. A., N. A. Fox, and M. R. Gunnar. 2011. "Same Place, Different Experiences: Bringing Individual Differences to Research in Child Care." *Child Development Perspectives* 5 (1): 44–49. doi:10.1111/j.1750-8606.2010.00155.x.

Ramberg, I. 2017. "- Det er utrolig gammeldags med bare tre dagers tilvenning." Adresseavisen, August 9. Accessed April 20, 2020. https://www.adressa.no/pluss/nyheter/2017/08/09/Det-er-utrolig-gammeldags-med-bare-tre-dagers-tilvenning-15127029.ece.

Sajaniemi, N., E. Suhonen, E. Kontu, P. Rantanen, H. Lindholm, S. Hyttinen, and A. Hirvonen. 2011. "Children's Cortisol Patterns and the Quality of the Early Learning Environment." *European Early Childhood Education Research Journal* 19 (1): 45–62. doi:10.1080/1350293X.2011.548938.

Salimetrics. 2019. "Collection Method: SalivaBio Children's Swab (SCS)." Accessed July 22, 2019. https://salimetrics.com/wp-content/uploads/2018/02/children-swab-saliva-collection-instructions.pdf.

Schore, J., and A. Schore. 2008. "Modern Attachment Theory: The Central Role of Affect Regulation in Development and Treatment." *Clinical Social Work Journal* 36 (1): 9–20. doi:10.1007/s10615-007-0111-7.

Shaffer, D. R., and K. Kipp. 2014. *Developmental Psychology. Child and Adolescence*. Belmont: Wadsworth.

Simonsson, M. 2015. "The Role of Artifacts During Transition Into the Peer Group: 1-to 3-Year-old Children's Perspective on Transition Between the Home and the Preschool in Sweden." *International Journal of Transitions in Childhood* 8: 14–24.

Statistics Norway. 2019a. "Befolkningens utdanningsnivå." Accessed December 19, 2019. https://www.ssb.no/utdanning/statistikker/utniv.

Statistics Norway. 2019b. "Household income, by type of household. Number of households and median (C) 2005–2018." Accessed April 2, 2020. https://www.ssb.no/97014/median-inntekt-etter-skatt-etter-fylke-og-husholdningstype.kroner.

Statistics Norway. 2019c. "Innvandrere og norskfødte med innvandrerforeldre." Accessed December 19, 2019. https://www.ssb.no/befolkning/statistikker/innvbef.

Statistics Norway. 2020. "Kindergarten." Accessed April 7, 2020. https://www.ssb.no/en/utdanning/statistikker/barnehager.

Suhonen, E., N. K. Sajaniemi, A. Alijoki, and M. A. Nislin. 2018. "Children's Biological Givens, Stress Responses, Language and Cognitive Abilities and Family Background After Entering Kindergarten in Toddlerhood." *Early Child Development and Care* 188 (3): 345–358. doi:10.1080/03004430.2016.1218157.

Sumner, M. M., K. Bernard, and M. Dozier. 2010. "Young Children's Full-Day Pattern of Cortisol Production on Child Care Days." *Archives of Psychiatric & Adolescent Medicine* 164 (6): 567–571. doi:10.1001/archpediatrics.2010.85.

Tervahartiala, K., L. Karlsson, J. Pelto, S. Kortesluoma, S. Hyttinen, A. Ahtola, N. Junttila, and H. Karlsson. 2019. "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 & Adolescent Psychiatry*. Advance online publication. doi:10.1007/s00787-019-01432-3.

Tribble, R. C., J. Dmitrieva, S. E. Watamura, and M. K. LeBourgeois. 2015. "The Cortisol Awakening Response (CAR) in Toddlers: Nap-dependent Effects on the Diurnal Secretory Pattern." *Psychoneuroendocrinology* 60: 46–56. doi:10.1016/j.psyneuen.2015.05.009.

Undheim, A. M., and M. B. Drugli. 2012. "Perspective of Parents and Caregivers on the Influence of Full-time Day-care Attendance on Young Children." *Early Child Development and Care* 182 (2): 233–247. doi:10.1080/03004430.2011.553678.

Vermeer, H. J., and M. G. Groeneveld. 2017. "Children's Physiological Responses to Childcare." *Current Opinion in Psychology* 15: 201–206. doi:10.1016/j.copsyc.2017.03.006.

Vermeer, H. J., M. G. Groeneveld, I. Larrea, M. H. van IJzendoorn, A. Barandiaran, and M. Linting. 2010. "Child Care Quality and Children's Cortisol in Basque Country and the Netherlands." *Journal of Applied Developmental Psychology* 31 (4): 339–347. doi:10.1016/j.appdev.2010.05.001.

Vermeer, H. J., and M. H. van Ijzendoorn. 2006. "Children's Elevated Cortisol Levels at Daycare: A Review and Meta-analysis." *Early Childhood Research Quarterly* 21 (3): 390–401. doi:10.1016/j.ecresq.2006.07.004.

Watamura, S. E., C. L. Coe, M. L. Laudenslager, and S. S. Robertson. 2010. "Child Care Setting Affects Salivary Cortisol and Antibody Secretion in Young Children." *Psychoneuroendocrinology* 35 (8): 1156–1166. doi:10.1016/j.psyneuen.2010.02.001.

Watamura, S. E., B. Donzella, D. A. Kertes, and M. R. Gunnar. 2004. "Developmental Changes in Baseline Cortisol Activity in Early Childhood: Relations with Napping and Effortful Control." *Developmental Psychobiology* 45 (3): 125–133. doi:10.1002/dev.20026.

Appendix

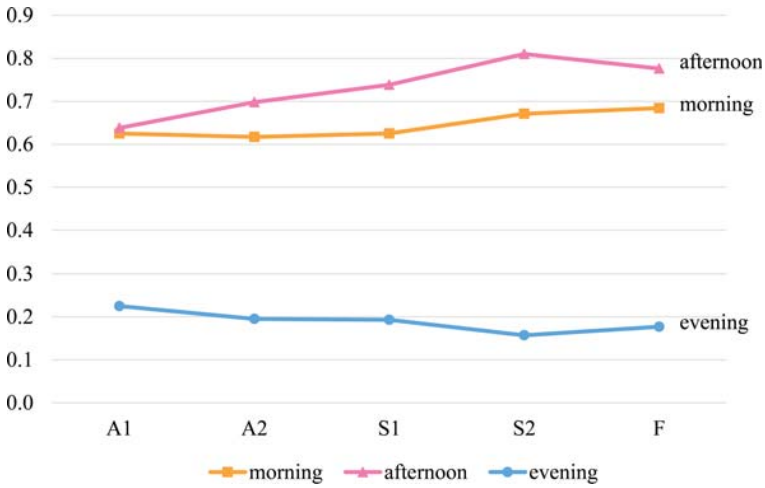


Figure A1. Estimated marginal means of cortisol in nmol/l (log10-transformed) for girls.

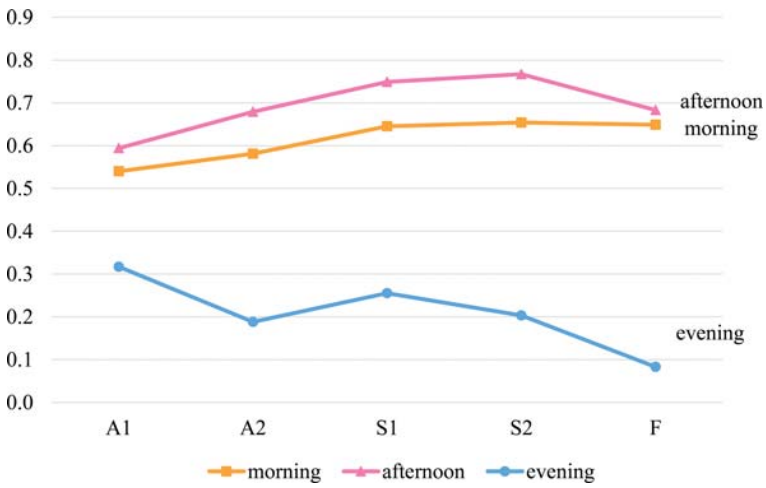


Figure A2. Estimated marginal means of cortisol in nmol/l (log10-transformed) for boys.

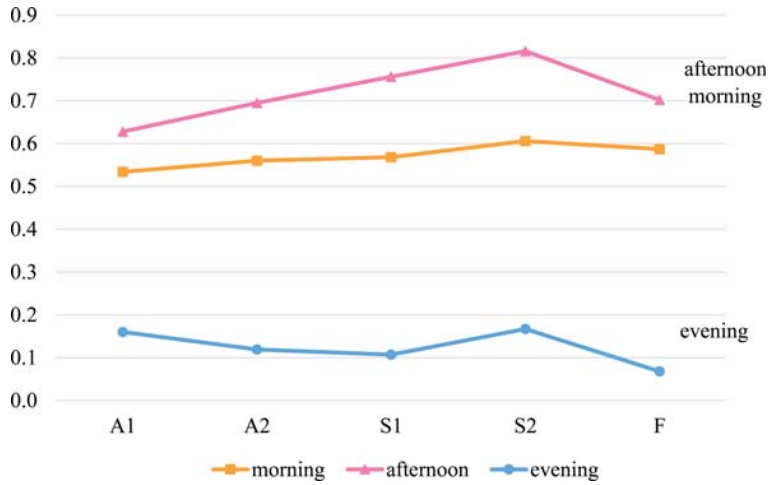


Figure A3. Estimated marginal means of cortisol in nmol/l (log10-transformed) for children with no siblings.

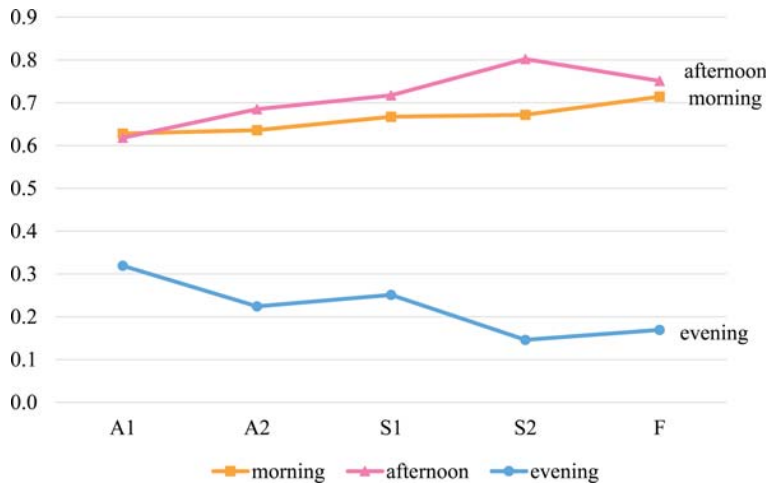


Figure A4. Estimated marginal means of cortisol in nmol/l (log10-transformed) for children with siblings.

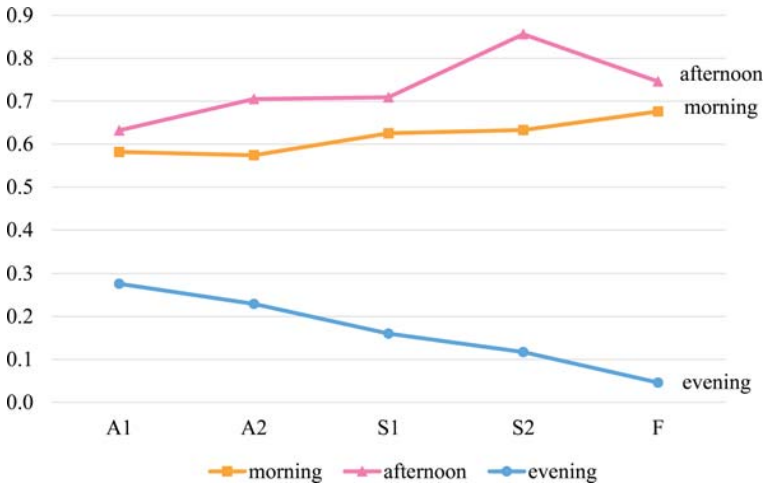


Figure A5. Estimated marginal means of cortisol in nmol/l (log10-transformed) for children in child-care groups with up to 11 children.

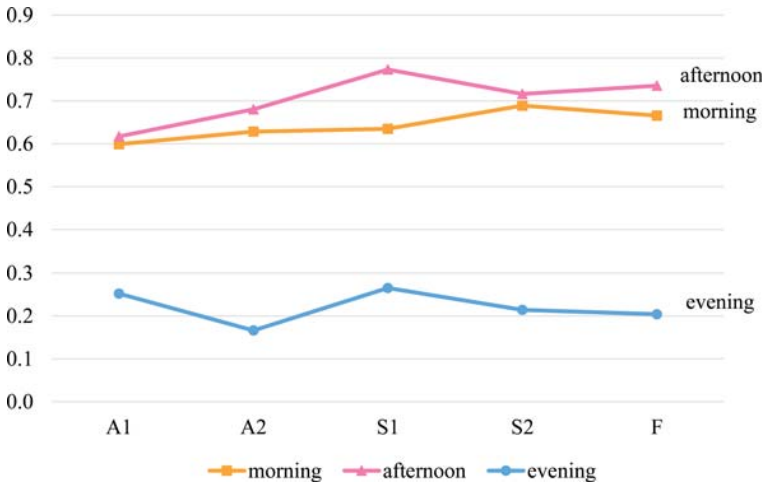


Figure A6. Estimated marginal means of cortisol in nmol/l (log10-transformed) for children in child-care groups with more than 11 children.

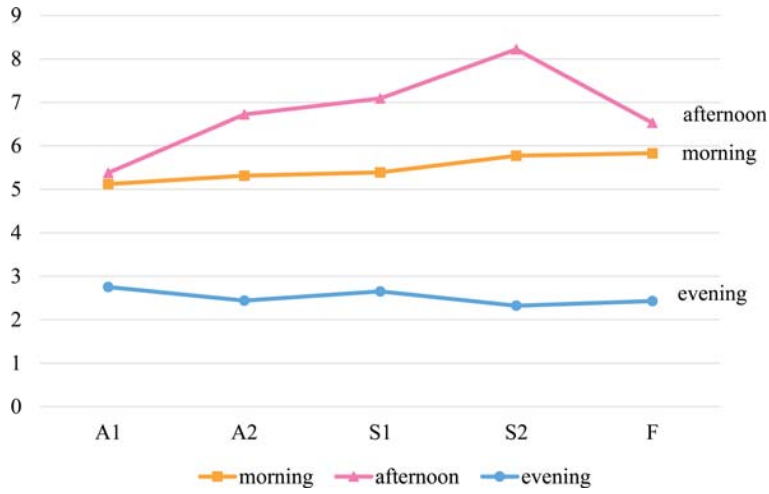


Figure A7. Mean untransformed cortisol levels in nmol/l.

Paper 2

Toddlers' Cortisol Levels in Childcare and at Home

Kathrin Nystad[1], May Britt Drugli[1,2], Stian Lydersen[1], Håvard Horndalen Tveit[3], Ratib Lekhal[4,5], and Elisabet Solheim Buøen[6]

[1] Regional Centre for Child and Youth Mental Health and Child Welfare, Faculty of Medicine and Health Science, Department of Mental Health, Norwegian University of Science and Technology (NTNU)

[2] Centre of the Study of Educational Practice, Inland Norway University of Applied Sciences

[3] HUNT Research Center, Faculty of Medicine and Health Science, Department of Public Health and Nursing, Norwegian University of Science and Technology (NTNU)

[4] Department of Communication and Culture, Norwegian Business School, Oslo

[5] Department of Education, University of Oslo

[6] Regional Centre for Child and Adolescent Mental Health Eastern and Southern Norway

Author Note

Kathrin Nystad: https://orcid.org/0000-0003-0844-3642

May Britt Drugli: https://orcid.org/0000-0002-2325-8552

Stian Lydersen: https://orcid.org/0000-0001-6613-8596

Håvard Horndalen Tveit: https://orcid.org/0000-0003-3262-761X

Ratib Lekhal: https://orcid.org/0000-0002-3750-4275

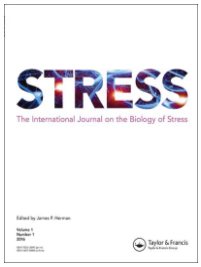
Elisabet Solheim Buøen: https://orcid.org/0000-0001-7120-4586

We have no known conflict of interest to disclose.

Correspondence regarding this article should be addressed to Kathrin Nystad, Regional Centre for Child and Youth Mental Health and Child Welfare, Norwegian University of Science and Technology (NTNU), Trondheim, Norway. Postal address: Regional Centre for Child and Youth Mental Health and Child Welfare, P.O. Box 8905, MTFS, N-7491 Trondheim

Email: kathrin.nystad@ntnu.no

Paper 3



Stress
The International Journal on the Biology of Stress

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ists20

Change in toddlers' cortisol activity during a year in childcare. Associations with childcare quality, child temperament, well-being and maternal education

Kathrin Nystad, May Britt Drugli, Stian Lydersen, Ratib Lekhal & Elisabet Solheim Buøen

To cite this article: Kathrin Nystad, May Britt Drugli, Stian Lydersen, Ratib Lekhal & Elisabet Solheim Buøen (2022) Change in toddlers' cortisol activity during a year in childcare. Associations with childcare quality, child temperament, well-being and maternal education, Stress, 25:1, 156-165, DOI: 10.1080/10253890.2022.2048371

To link to this article: https://doi.org/10.1080/10253890.2022.2048371

Published online: 07 Apr 2022.

STRESS
2022, VOL. 25, NO. 1, 156–165
https://doi.org/10.1080/10253890.2022.2048371

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Change in toddlers' cortisol activity during a year in childcare. Associations with childcare quality, child temperament, well-being and maternal education

Kathrin Nystad[a], May Britt Drugli[a]*, Stian Lydersen[a], Ratib Lekhal[b#] and Elisabet Solheim Buøen[b,c]

[a]Regional Centre for Child and Youth Mental Health and Child Welfare, Department of Mental Health, Faculty of Medicine and Health Science Norwegian University of Science and Technology (NTNU), Trondheim, Norway; [b]Department of Communication and Culture, Norwegian Business School, Oslo, Norway; [c]Regional Centre for Child and Adolescent Mental Health Eastern and Southern Norway, Oslo, Norway

ABSTRACT

Elevated levels of the stress hormone cortisol have been found in toddlers in childcare. Measuring cortisol may provide an indication of children's experiences in childcare and help to adjust practices better to their needs. To the best of our knowledge, toddlers' cortisol levels in childcare have not yet been investigated longitudinally. Furthermore, it is unclear which child and childcare factors contribute to cortisol elevation in toddlers. Using linear mixed model analyses, we investigated the full-day cortisol activity (10.00 h, 15.00 h, 18.00 h) of 156 toddlers (81 female, 56 male) during a year in childcare (September, January, June). We also investigated child cortisol levels at home in January. In addition, we tested the relation between cortisol activity and changes in cortisol activity across the year and childcare quality, temperament, well-being in childcare, and maternal education. We found increasing evening cortisol levels through the year while controlling for age. Afternoon cortisol levels were stable, but above morning cortisol levels in September and January and only slightly below morning cortisol levels in June. At home in January, afternoon levels were significantly below morning levels. Higher well-being in childcare was associated with lower overall cortisol levels and less increase in evening cortisol levels through the year in childcare. Further, less active toddlers seemed to accumulate some stress during the childcare day, indicated by higher evening cortisol levels. Rising evening cortisol levels may indicate accumulating stress across the year. Results point toward childcare being demanding for toddlers and their need for consideration from caregivers and parents, also after a longer period of childcare attendance. The findings underline the importance of observing, promoting, and further researching children's well-being in childcare.

ARTICLE HISTORY
Received 22 June 2021
Accepted 22 February 2022

KEYWORDS
Toddler; childcare; cortisol; well-being; longitudinal; Thrive by Three

Introduction

Measuring the stress hormone cortisol in saliva may give an indication of children's experience in childcare (Vermeer & Van Ijzendoorn, 2006) which in turn can inform how to adjust childcare practice to meet children's needs in better ways. One- and two-year-old children (toddlers) have shown elevated levels of cortisol when in childcare compared to home (Drugli et al., 2018; Groeneveld et al., 2010; Legendre, 2003; Sumner et al., 2010; Watamura et al., 2003). Meta-analytic evidence suggests that they do so more than other age groups (Vermeer & Van Ijzendoorn, 2006).

Cortisol is a hormone produced by the hypothalamic-pituitary-adrenal axis (HPA). Cortisol levels both follow a basal daily rhythm, high around awakening and then declining across the day, and under conditions of stress or threat can elevate above basal levels (Gunnar & Herrera, 2013). A daily pattern with mid-afternoon cortisol levels not being higher than mid-morning levels has been observed in children from about 12 months (Gunnar & Donzella, 2002; Watamura et al., 2004). Having a balanced diurnal cycle of cortisol is important for individuals' development and health (Gunnar & Herrera, 2013). The HPA axis releases additional cortisol if an overly demanding situation is encountered. Rising cortisol levels are recognized as an indicator of social stress (Vermeer & Van Ijzendoorn, 2006).

While short and minor cortisol elevations may be beneficial and necessary for learning (Center on the Developing Child, 2021; Suhonen et al., 2018), it is unclear how small daily elevations over a long period impact children's functioning and development (Gunnar & Herrera, 2013). Chronic cortisol elevations have been observed to inhibit children's immune system (Watamura et al., 2010) and cognitive development (Phillips et al., 2011), and increase their vulnerability to future stress (Loman & Gunnar, 2010).

CONTACT Kathrin Nystad kathrin.nystad@ntnu.no Regional Centre for Child and Youth Mental Health and Child Welfare, Department of Mental Health, Faculty of Medicine and Health Science Norwegian University of Science and Technology (NTNU), Trondheim, 7491, Norway
*Centre of the Study of Educational Practice, Inland Norway University of Applied Sciences, Hamar, Norway.
#Department of Education, University of Oslo, Oslo, Norway.

To the best of our knowledge, toddlers' cortisol activity has not yet been investigated longitudinally through a year in childcare. Measuring the amount and movements of cortisol through a year may help identify challenging phases or tendencies of habituation or stress accumulation. Cortisol levels in childcare are likely a function of both child and childcare characteristics (Vermeer & Groeneveld, 2017). We do not know enough about the contexts in which toddlers experience stress in childcare or if there are groups of children who are more exposed to cortisol elevations than others and hence in greater need of consideration from caregivers. It has been recognized that individual children experience childcare differently (Phillips et al., 2011), and that care contexts vary in their content and quality (Cadima et al., 2020). Cortisol activity in childcare may be linked to process quality (Vermeer & Groeneveld, 2017) and structural quality of care (Legendre, 2003), child temperament (Dettling et al., 2000; Gunnar et al., 2011), child well-being (Groeneveld et al., 2010) as well as family SES (Berry et al., 2014). However, research findings regarding these factors are not conclusive when it comes to toddlers (Drugli et al., 2018; Groeneveld et al., 2010; Ouellet-Morin et al., 2010; Suhonen et al., 2018; Sumner et al., 2010; S. Watamura et al., 2003) and they need to be studied further.

To explore cortisol activity through the day as well as changes in cortisol activity across a year, we assessed toddlers' cortisol levels in the morning and afternoon in childcare as well as in the evening at home during one year in childcare, with measurements on two subsequent days in September, January and June. We also studied how process and structural quality in childcare, child temperament, well-being in childcare, and maternal education were related to daily cortisol activity and changes in cortisol activity during the year.

We expected afternoon cortisol to be higher and evening cortisol to be distinctly lower than morning cortisol on all measurement days (Sumner et al., 2010; Vermeer & Van Ijzendoorn, 2006). Due to a lack of research, we did not form a hypothesis regarding changes in cortisol activity across the year. Lower childcare quality (Legendre, 2003; Vermeer & Groeneveld, 2017), inhibited or negative temperament (Dettling et al., 2000; Watamura et al., 2003) and lower well-being in childcare (Groeneveld et al., 2010) was expected to be related to higher overall cortisol levels and higher afternoon elevations. We did not form a hypothesis regarding maternal education due to a lack of previous research.

Methods

Thrive by three

This study was conducted on a subsample of a large Norwegian childcare study called Thrive by Three. Thrive by Three is a cluster-randomized control trial that seeks to investigate the effects of an extensive quality-building program (Thrive by Three) aimed at improving the quality of caregiver–toddler interactions (i.e. process quality) and explores the effects of process quality on children's development and mental health (Lekhal et al., 2020). The present study was conducted on a sample of children from childcare centers in the control group.

Childcare centers in seven Norwegian municipalities had the possibility of opting into Thrive by Three. A total of 78 centers participated in the study, and 794 caregivers consented to fill out questionnaires about themselves and the children. Caregivers informed parents about Thrive by Three, and parents were able to enroll their child into the study. Parents gave informed consent for their child to participate. Where custody was shared, both guardians had to consent for the child to participate in the study. We randomly chose four to five childcare centers from each municipality. Children with valid consent were randomly selected for saliva sampling. No more than 13 children were sampled from any given childcare center. A selection of 157 children gave saliva samples for the present study to investigate their cortisol levels. A flow chart on participant recruitment of the present study can be found in Figure 1. Thrive by Three, including the present study, was approved by the Regional Committee for Medical and Health Research Ethics and the Norwegian Center for Research Data.

Saliva sampling

Saliva was sampled in the beginning (T1 = September), middle (T2 = January), and end (T3 = June) of the childcare year 2018/2019. Each sampling point consisted of two sequential days. Caregivers collected samples in the morning and afternoon in childcare. Parents sampled saliva in the evening at home. In addition, saliva was sampled at home in January, in the morning and afternoon on two sequential weekend days. We did not collect evening samples at T2. Overall, saliva sampling was scheduled for 159 children at 20 time points, with two children dropping out of the study before saliva sampling.

The Salimetrics' SalivaBio Children's Swab was used to collect saliva. This tool is designed to sample saliva from children and has been validated for cortisol analysis (Salimetrics, 2019). Parents and caregivers were instructed to apply a playful method of sampling to make the procedure less invasive

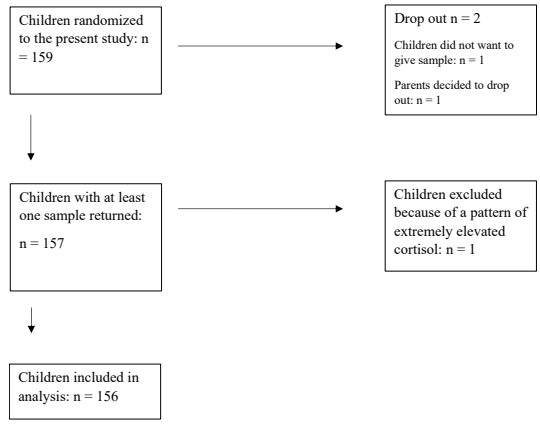


Figure 1. Flow chart of participant recruitment.

for children. No stimulant was used. Children who refused to give a sample were not compelled to do so. We froze samples at −20 °C and sent them to the cortisol laboratory at the University of Trier, Germany. Saliva was analyzed for cortisol using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA) (Dressendörfer et al., 1992). All samples were destroyed after analysis.

We received 2641 (83.1%) of the 3180 possible samples. The laboratory excluded 200 (6.3%) of those samples because the test tubes contained too little saliva for immunoassay. We excluded a further 77 (2.4%) samples because of an exceedingly high cortisol level or illogically low level ($n = 1$). All samples from one individual were excluded because of a constant pattern of extremely high values. Based on guidelines from both the laboratory at the University of Trier and at St. Olav's Hospital in Trondheim, we assumed that values greater than 30 nmol/l reflected sickness, medication, or contamination and removed them from the analysis. 2364 (74.4%) cortisol values from 156 children enrolled in 31 different childcare groups in 13 childcare centers were available for statistical analyses. Most children had one or several samples missing. Sixteen children had a complete set of 20 samples. Table 1 and Figure 2 give an overview of the untransformed mean cortisol values.

Predictors of cortisol activity

We also investigated the relationship between cortisol activity and a row of predictors measured at T1.

Childcare quality

Head teachers answered electronic questionnaires at T1 on the number of children and number of caregivers with a bachelor's degree (pedagogs) in their group.

Process quality refers here to quality of caregiver-child interactions and was assessed at the group level with the Classroom Assessment Scoring System (CLASS) Toddler (La Paro et al., 2012) at T1. CLASS Toddler measures the quality of interactions between children and caregivers in toddler childcare on two domains with eight dimensions: Domain 1, *Emotional and Behavioral Support (EBS)*, contains the dimensions *Positive Climate*, *Negative Climate*, *Teacher Sensitivity*, *Regard for Child Perspective* and *Behavioral Guidance*. Domain 2, *Engaged Support for Learning (ESL)*, consists of the dimensions *Facilitation of Learning and Development*, *Quality of Feedback* and *Language Modeling*. Pakarinen et al. (2010) and Slot et al. (2017) found evidence for the criterion-based validity of CLASS and concluded that the tool applies to measure quality in childcare in a context outside the US. The Cronbach's alpha in the present study was 0.89 for EBS and 0.95 for ESL. Observers were certified after a two-day training and scoring of five training videos, where they had to reach at least 80% agreement with the master codes. A day-long refreshment session was organized right before the observations took place. The interrater reliability Intraclass Correlation Coefficient in the Thrive by Three main study was 0.88 for EBS and 0.91 for ESL. Groups were observed for three loops of 15 minutes on one morning. Each loop was scored individually on a 7-point Likert scale, and subsequently an average score for each dimension was calculated. Average dimensional scores were averaged into a domain score for EBS and ESL. A score of 1–2 represents low quality, 3–5 middle range quality, and 6–7 high quality (La Paro et al., 2012). Information on the childcare groups' structural factors and process quality scores can be found in Table 2.

Temperament

Child temperament was assessed using the Emotionality Activity Sociability Temperament Survey (EAS) (Buss & Plomin, 1984). The EAS is a short instrument with 20 items,

Table 1. Descriptive statistics of untransformed cortisol.

Month	Time	*n*	Mean	SD	Median	Minimum	Maximum
September	Morning 1	137	4.91	3.95	3.92	0.99	23.88
	Afternoon 1	129	5.32	3.62	4.22	0.43	21.84
	Evening 1	132	2.58	4.08	1.19	0.01	25.45
	Morning 2	127	5.60	4.41	4.47	0.61	27.42
	Afternoon 2	124	6.18	4.62	4.91	0.70	26.45
	Evening 2	124	2.40	3.52	1.25	0.01	22.12
January	Morning 1	133	5.54	4.48	4.22	0.38	25.31
	Afternoon 1	126	5.51	3.29	4.79	0.44	21.89
	Morning 2	127	4.59	3.10	3.94	0.68	26.32
	Afternoon 2	118	5.16	3.62	4.03	0.86	21.61
Home	Morning 1	109	4.95	4.55	3.69	0.15	29.05
	Afternoon 1	103	4.17	4.72	2.82	0.28	29.13
	Morning 2	102	5.10	4.40	3.95	0.64	26.86
	Afternoon 2	98	4.00	4.71	2.32	0.065	24.28
June	Morning 1	131	4.82	3.53	4.18	1.09	27.48
	Afternoon 1	125	4.71	3.28	3.94	1.04	22.59
	Evening 1	92	2.17	3.46	1.20	0.02	22.18
	Morning 2	123	4.71	3.51	3.79	0.67	29.26
	Afternoon 2	109	4.88	4.40	3.55	0.72	28.35
	Evening 2	95	2.37	3.11	1.33	0.07	17.20
Valid *n* (listwise)		16					

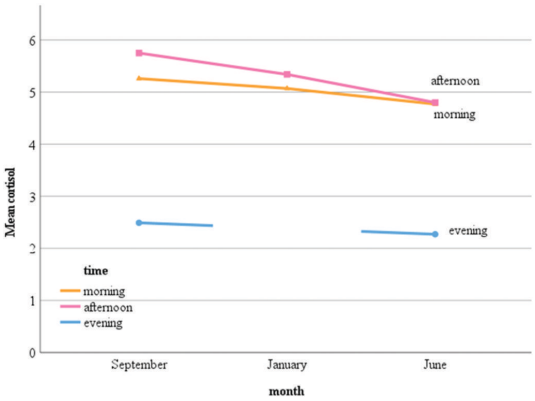


Figure 2. Mean untransformed cortisol in nmol/l.

Table 2. Information on childcare group quality for the 31 childcare groups.

	n	Mean	SD	Min	Max
CLASS: EBS	31	5.58	1.04	3	7
CLASS: ESL	31	3.37	1.12	1.2	6.13
Group size	27	10.93	1.94	8	16
Pedagogue ratio	26	0.41	0.13	0.25	0.75
Valid *n* (listwise)	26				

e.g. "This child prefers playing with others rather than alone," on a 5-point Likert scale (1 = very typical, 5 = not typical) and a structure of four factors: Shyness, Emotionality, Activity and Sociability. A higher sum score indicates a higher prevalence of the temperamental trait. We used the EAS ratings from mothers at T1. Mathiesen and Tambs (1999) undertook a psychometric investigation of the EAS in a sample of Norwegian toddlers and confirmed a four-factor structure, adequate stability over time, suitability for the toddler age group, and moderate internal consistency, with Cronbach's alpha ranging from 0.48 to 0.71 for children aged 18 months. Internal consistency was rising with age. In the present study, Cronbach's alpha was 0.71 for the Shyness scale, 0.79 for the Emotionality scale, 0.77 for the Activity scale, and 0.60 for the Sociability scale. Because of a low Cronbach's alpha, the Sociability scale was removed from further analysis.

Child well-being in childcare

Children's well-being in childcare was measured with the Leiden Inventory for the Child's Well-being in Daycare (LICW-D) (De Schipper, Van Ijzendoorn et al., 2004). The inventory consists of 12 items concerning children's relationships to caregivers, peers and surroundings in childcare, e.g. "this child is actively seeking contact with other children", rated on a 6-point Likert scale (1 = applies never, 6 = applies always), loading onto the factor "this child enjoys attending the childcare center". The Norwegian translation was adjusted to a 5-point Likert scale, as there was no meaningful linguistic differentiation between "4 = applies regularly" and "5 = applies often" (Van Trijp et al., 2021). Caregivers answered the LICW-D at T1. Mean scores were calculated with a higher score indicating higher well-being. The instrument has shown good internal consistency (Cronbach's alpha = 0.81) (De Schipper, Van Ijzendoorn et al., 2004). In in the current study, Cronbach's alpha was 0.87. A psychometric evaluation of the LICW-D by Van Trijp et al. (2021) confirmed the appropriate construct and concurrent validity and applicability for toddlers in Norwegian childcare centers.

Socioeconomic status

Parents answered electronic questionnaires on household income, marital status, education, and language background at T1. We investigated the relationships between cortisol levels and maternal education (university education vs. no university education). Demographic information on the participating families can be found in Table 3. Families who participated in the present study were predominantly Norwegian and middle class (Statistics Norway, 2019, 2020, 2021).

Statistical analysis

To analyze the effect of month and time of day on cortisol levels, we applied a linear mixed model, with cortisol (in nmol/l, log10-transformed) as the dependent variable and individual as random effect. The month of measurement was entered as a three-category covariate. Each month had two sequential measurement days, which were coded as the same day, as there was no qualitative difference between them. Also, time of day (morning, afternoon, evening) was entered as a three-category covariate. We included the interaction between month and time of day. Additionally, we controlled for age and gender. Gender had no effect on the model and was therefore excluded from analysis. Age had a significant effect ($p < 0.001$) and was therefore controlled for in all further analyses. A linear mixed model includes all available data in the estimation, so there was no need to impute missing values.

In addition, we conducted a robustness check and examined cortisol measurement from samples measured at home on Saturday and Sunday in January, using a linear mixed model, with cortisol (in nmol/l, log10-transformed) as the dependent variable, individual as random effect, and afternoon versus morning as covariate. This analysis was done to assure that our results in fact reflected child cortisol levels in the childcare setting, and not a general pattern of change of child cortisol during the day.

To analyze the relation between process quality, group size, number of pedagogs in each group, child temperament, well-being and maternal education and cortisol activity, we entered those additional variables one at a time as a covariate with their two- and three-way interactions with time of day and month into our initial mixed model, while controlling for age. Normality of residuals was checked by visual inspection of Q-Q plots. When controlling for age, we found slight deviations from normality. In this case, we also carried out the analysis using bootstrapping with B = 2,000 bootstrap replications and the bias corrected and accelerated method (BCa). The bootstrapped analysis results were substantially the same as those for the non-bootstrapped analysis (data not shown).

Due to multiple hypotheses, we regarded two-sided p-values <0.01 as statistically significant. We and report 95% confidence intervals where relevant.

All analyses were carried out using SPSS27.

Table 3. Demographic information (mother $n = 155$, missing = 20; father $n = 149$, missing = 54).

	n mother (%)	*n* father (%)
Language background		
Norwegian	116 (86.0)	84 (88.4)
Europe or North America	9 (6.6)	5 (5.3)
Other	10 (7.4)	6 (6.3)
Marital status		
In a relationship	127 (94.1)	94 (98.9)
Single	8 (5.9)	1 (1.1)
Education		
Elementary school (9–10 years)	1 (0.7)	4 (4.2)
Finished secondary school	25 (18.5)	25 (26.3)
Up to 4 years with university education	44 (32.6)	28 (29.5)
More than 4 years of university education	65 (48.2)	38 (40.0)
Annual household income before tax		
Under 200,000 NOK*	3 (2.2)	1 (1.1)
200,000-599,000 NOK	14 (10.4)	14 (14.7)
600,000-999,000 NOK	49 (36.3)	26 (27.4)
More than 1,000,000 NOK	69 (51.1)	54 (56.8)

*In 2019, 1 NOK equaled approximately 0.1 USD.

Results

Cortisol activity through the day and changes in cortisol activity through the year in childcare

To investigate the daily cortisol activity and changes in cortisol activity across the year, we compared the difference in

cortisol levels through time of day (morning, afternoon, evening) and year (September, January, June) while controlling for age. Estimated marginal means and number of observations can be found in Table 4.

Cortisol activity through the day

Morning cortisol was set as a reference point. Afternoon cortisol was significantly higher than morning cortisol in September. Untransformed cortisol rose 8.4% between the morning and afternoon on the first measurement and 10.4% on the second day of measurement in September. Afternoon cortisol did not differ significantly from morning cortisol in January and June. Evening cortisol was significantly lower than morning cortisol at each point of measurement. Figure 3 shows the estimated marginal means and corresponding p-values.

Changes in cortisol activity through the year

Evening cortisol increased significantly between September and June. There was no significant change in morning and afternoon cortisol during the year. Figure 3 gives an overview of the estimated marginal means and p-values when comparing morning, afternoon, and evening cortisol levels through the year.

Cortisol activity through the day at home

In the weekend in January, the mean log10 cortisol level was reduced from 0.589 in the morning to 0.406 in the afternoon, mean difference −0.183, 95% CI (−0.245 to −0.121), $p < 0.001$. The estimated means are shown in Figure 3.

Relation between cortisol activity and changes in cortisol activity and child and childcare factors

For our second research question, we entered the additional variables one by one as covariates with two- and three-way interactions with time of day and month into the mixed model while controlling for age. For information on child factors, see Table 5.

Children's well-being in childcare ($p < 0.001$) and the EAS activity scale ($p = 0.009$) were significantly related to the dependent variable cortisol, while CLASS scores (EBS, ESL), group size, number of pedagogs with a bachelor's degree in the group, other aspects of child temperament (Shyness, Emotionality), and maternal education were not found to be related to cortisol levels. Both daily cortisol activity and changes in cortisol activity across the year differed according to well-being scores. Daily cortisol activity differed according to activity scores. Figures 4 and 5 show the estimated marginal means of cortisol levels controlled for age for children with a well-being score below and above the median (=4.33). Figures 6 and 7 show the estimated marginal means of cortisol levels controlled for age for children with an activity score below and at as well as above the median (=20.00).

Discussion

In the present study, we measured cortisol activity during the day as well as changes in cortisol activity through a year in childcare. We also investigated the relationship between cortisol activity and changes in cortisol activity and childcare quality, child temperament, well-being, and maternal

Table 4. Estimated marginal means of log10-transformed cortisol in childcare.

Month	Time	n	Mean	95% Confidence Interval		
September	Morning	264	0.576	0.530	to	0.622
	Afternoon	253	0.637	0.590	to	0.684
	Evening	256	0.071	0.024	to	0.117
January	Morning	260	0.622	0.579	to	0.665
	Afternoon	244	0.659	0.615	to	0.704
	Evening					
June	Morning	254	0.652	0.605	to	0.699
	Afternoon	234	0.637	0.589	to	0.686
	Evening	187	0.187	0.134	to	0.239

Table 5. Descriptive statistics of child characteristics.

	n	%/Mean	SD	Min	Max
Gender	156	–	–	–	–
Female	81	51.9	–	–	–
Male	75	48.1	–	–	–
Age in months at T1	145	21.75	6.31	10	32
EAS Shyness	132	12.11	3.05	5	22
EAS Emotionality	133	14.08	3.31	6	23
EAS Activity	134	19.76	2.98	13	25
LICW-D	145	4.26	0.49	2.83	5.00
Valid n (listwise)	125	–	–	–	–

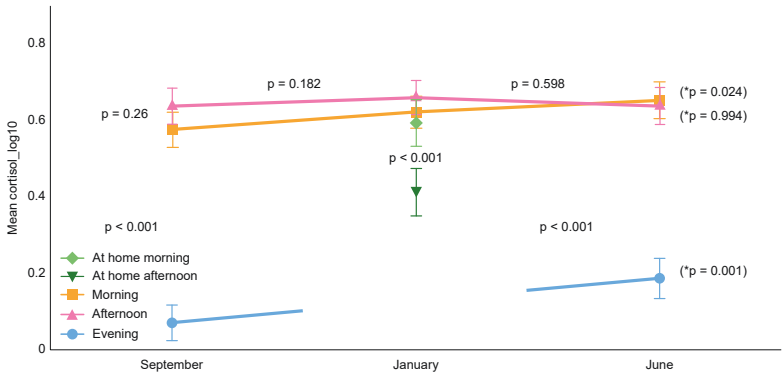


Figure 3. Estimated marginal means of log10-transformed cortisol (nmol/l) with p-values for change compared to morning and *p*-values (*) for change compared to September.

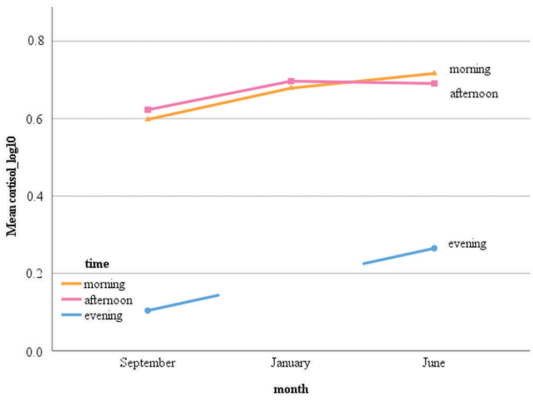


Figure 4. Estimated marginal means of log10-transformed cortisol (nmol/l) in childcare for children with a well-being score below the median.

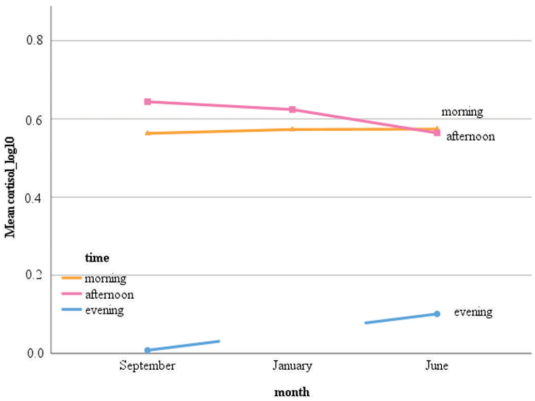


Figure 5. Estimated marginal means of log10-transformed cortisol (nmol/l) in childcare for children with a well-being score above the median.

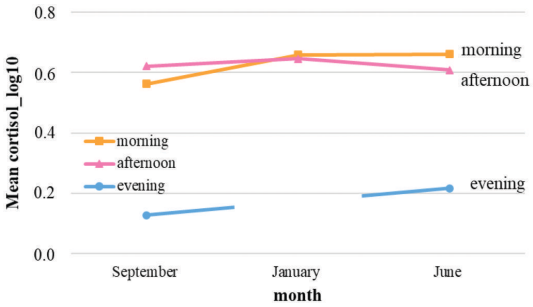


Figure 6. Estimated marginal means of log10-transformed cortisol (nmol/l) in childcare for children with an activity score below the median.

education. At one timepoint (January) we also analyzed child cortisol levels at home. As far as we are aware, the present study is the only study so far to longitudinally investigate toddlers' cortisol activity in childcare. Cortisol levels varied significantly with the time of day and month. Further, change in cortisol levels showed another profile at home than in

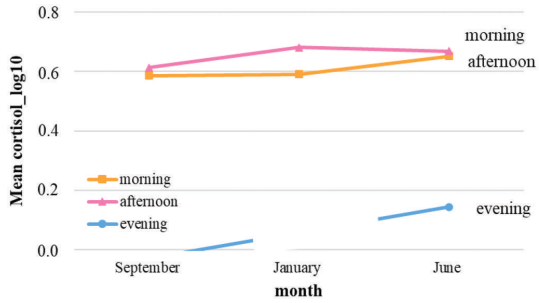


Figure 7. Estimated marginal means of log10-transformed cortisol (nmol/l) in childcare for children with an activity score at or above the median.

childcare. Well-being and child temperament in terms of activity level were the only independent variables found to be related to cortisol activity and changes in cortisol activity. Due to a lack of longitudinal research with toddlers, we compare our findings to the few studies with preschoolers or infants as participants.

Main finding: Cortisol activity through the day and changes in cortisol activity through the year

Cortisol activity through the day

Afternoon cortisol levels were significantly higher than morning cortisol levels in September. Contrary to our expectations, afternoon cortisol levels did not differ from morning cortisol levels in January and June. Cortisol levels did not follow the diurnal pattern from morning to afternoon, as it has been observed mostly observed at home (Vermeer & Van Ijzendoorn, 2006), at any of the measurement points. Afternoon cortisol levels may therefore reflect activation in childcare through the year. This assumption is supported both by our present measurement at home in January and by a previous study among Norwegian toddlers where afternoon levels of cortisol were high on childcare days, while they were low on days when children stayed at home (Drugli et al., 2018).

Evening cortisol levels were significantly lower than morning cortisol levels at all points of measurement. Children seemed to unwind at home after childcare. The home environment may be less challenging, and parents have been observed to give sensitive care to their toddlers after a day in childcare (Ahnert et al., 2000), which is a potent regulator of the HPA axis (Gunnar & Donzella, 2002). Decreasing cortisol levels toward the evening may indicate that there is no extended stress activation (Nystad et al., 2021). Only a few studies have investigated toddlers' evening cortisol levels at home after childcare: Sumner et al. (2010), Groeneveld et al. (2010) and Nystad et al. (2021) also observed a decrease in evening cortisol levels.

Changes in cortisol activity through the year

In the present study evening cortisol increased significantly across the childcare year, a pattern also found in preschoolers (Sajaniemi et al., 2014). This indicates that children's stress activation in childcare increasingly spills over to the

home setting as the year proceeds. As stress accumulates across the year, the children may be less able to unwind at home in the evening at the end of the year. This in turn may be due to a change in parental care practices at home after childcare. Parents have been observed to provide soothing care to their toddlers at home after childcare (Ahnert et al., 2000). Sensitive parental care is an important regulator of the HPA axis (Gunnar & Donzella, 2002). Parents' attention might have diminished across the year as children got older and parents became more relaxed about childcare attendance, which in turn may have resulted in rising evening levels of cortisol. Also, seasonal changes could have played a role, and the summer months may be characterized by high activity for many families due to more free-time activities, light evenings, and warm temperatures.

The rising cortisol levels in the evening may reflect a general strain from childcare and indicate allostatic load. Blair et al. (2011) describe allostatic load as among other upregulations (downregulation in certain cases) of HPA axis activity as a reaction to accumulating stress, possibly interfering with appropriate hormonal reaction in the case of acute stress. This process has mainly been researched with children facing high socioeconomic risk (Evans, 2003; McEwen & Wingfield, 2003). It is unclear how moderate stressors, such as childcare attendance, contribute to allostatic load in young children. Baseline levels of cortisol have been observed to decrease during the first years of life (Blair et al., 2011; Watamura et al., 2004), which makes the observed increase in evening cortisol in childcare found in the present study noteworthy.

In summary, as the year proceeded, the children seemed to be less able to unwind in the evening. Thus, childcare attendance also seemed to be somewhat challenging for toddlers after a full year of participation. It is not clear if children habituate to childcare stressors (Gunnar & Herrera, 2013). Both parents and caregivers play an important role in regulating children's stress (Gunnar & Donzella, 2002). They should meet them with sensitivity and provide them with opportunities to settle down, especially in the afternoon in childcare (Undheim & Drugli, 2012) and the evening at home (Ahnert et al., 2000). And our findings indicate that toddlers may need such support from their parents through the first year in care, not just in the beginning.

Secondary finding: Child well-being, child temperament and cortisol activity and changes in cortisol activity

Child well-being in childcare was significantly related to cortisol activity and changes in cortisol activity. Measuring children's well-being, as well as their cortisol levels, allowed us to investigate children's experiences in childcare from the perspective of two informants: caregivers in childcare and children themselves. When it came to daily cortisol activity, children with lower well-being scores had higher overall cortisol levels at all points of measurement. When looking at changes in cortisol activity through the year, they showed a steeper incline in evening cortisol and an increase in morning and afternoon cortisol through the year. Children with higher well-being scores showed stable morning cortisol levels, declining afternoon cortisol levels, and only slightly increasing evening cortisol levels across the year. Differences seemed to be highest when it came to evening cortisol levels, where children with lower well-being scores showed both higher overall levels and a steeper incline across the year. The median for well-being was quite high, and we therefore compared children with very high well-being scores and others. There were no children with low well-being scores in the present study. To the best of our knowledge, the relationship between toddlers' well-being in childcare and their cortisol levels has only been tested once before. Groeneveld et al. (2010) did not find a relation between these factors. More research is therefore needed, and our findings need to be interpreted with caution.

Toddlers in our study with low activity levels were found to have higher evening cortisol levels at home both in the beginning and end of the childcare year as compared to toddlers with higher activity levels. Less active toddlers seem to accumulate some stress during the childcare day, which in turn is expressed through somewhat higher evening levels of cortisol at home. Low activity levels indicate among others that the child is moving slowly, is not very energetic and prefers quiet games (Buss and Plomin, 1984). Passive and quiet children may be in risk for being overlooked in childcare and not getting emotional support that buffers them from sources of social stress (Phillips, Fox and Gunnar, 2011). In a previous study, Watamura et al. (2003) found higher levels of stress among toddlers who were less involved with peer play. Probably, children with low activity levels need more responses and support from their caregivers than they get, for example to be able to be involved in positive interactions with other children.

The association between well-being, child temperament and cortisol activity and changes in cortisol activity strengthens the argument that measuring cortisol levels may give an indication of different children's experience in childcare (Vermeer & Van Ijzendoorn, 2006) and suggests that the observed rise in evening cortisol across the year may be related to childcare attendance. It also indicates the validity of caregivers' evaluations of children's experiences in childcare. Caregivers' ability to identify children who are in need of greater support should be trusted (Stensen et al., 2021). In the present study, child activity levels are reported by mothers, indicating that also parent involvement is important for being able to identify toddlers in risk for higher stress levels. We cannot make inferences about the causal direction of the relationship between cortisol levels and well-being and child activity levels. Children with high levels of well-being and high activity levels may experience less stress daily and less strain through the year in childcare. At the same time, children with lower-or better-regulated cortisol activity may show more well-being in childcare and higher activity levels. There may be third factors underlying both well-being, activity and cortisol levels, for example child and family characteristics as well as aspects of process quality (De Schipper et al., 2004; De Schipper, Van Ijzendoorn, et al., 2004; Groeneveld et al., 2010; Van Ijzendoorn et al., 1998; Van Trijp et al., 2021), although we were not able to find such relations in the present study.

It has been suggested that cortisol elevations in childcare could be positive stress, helping children to reach the next steps of development (Suhonen et al., 2018). Our finding that higher overall levels and a steeper incline of cortisol across the year were linked to lower well-being scores does not support this notion.

Strengths and limitations

An important strength of the present study is the higher number of participants than comparable studies (Albers et al., 2016; Groeneveld et al., 2010; Sajaniemi et al., 2014) and that saliva was sampled on two subsequent days in each month of measurement. This diminished the impact of both inter- and intra-individual variability of cortisol levels (Hanrahan et al., 2006). As one of few studies, we measured cortisol levels in the evening at home to assess children's full-day cortisol activity. As a robustness check, we also assessed cortisol levels during the weekend at home in January. This allowed us to expand our understanding of children's cortisol activity beyond the childcare context and thereby investigate potential connections between cortisol levels at home and in childcare.

There are certain limitations to the present study. Our study does not include a control group of children who did not attend childcare. We can therefore not be entirely sure that the observed daily and longitudinal changes in cortisol activity are produced by childcare. Seasonal variations of daylight exposure and developmental processes might also play a role. The children participating were mostly from Norwegian middle-class families with relatively high income and parental education. Families with a minority background were under-sampled. Children from backgrounds with socioeconomic risk may show different patterns of cortisol activity in childcare (Berry et al., 2014). Findings of the present study are limited to middle-class populations. Furthermore, we did not measure cortisol levels at home at all timepoints and therefore applied morning and September measurements as baseline. This prevents us from fully evaluating the significance of childcare attendance for the observed longitudinal changes in cortisol activity. We were not able to control for food-intake, morning wake-up times, nap times, and use of corticosteroids which might have altered cortisol levels either directly by stimulating the HPA axis or indirectly by interfering with immunoassay (Hanrahan et al., 2006; Tribble et al., 2015). Initial transition to childcare might have accounted for some afternoon cortisol elevation of certain children at the September measurement. However, all the children had spent at least four weeks in childcare when the first saliva sampling took place. We did not measure evening cortisol levels in January and could not know whether evening levels raised steadily through the year or spontaneously between the middle and end of the year, which limits our ability to identify potential reasons for the observed incline in evening cortisol levels. Even if our study has relatively many participants, it may be under-powered. Recruitment of more participants could have enabled us to discover more differences in cortisol levels during the day and the year.

Implications and future research

Our findings may indicate a slight increase in fatigue among children as the year in childcare proceeds. Parents and caregivers should carefully consider children's individual needs in the second half of the year in childcare. Calm afternoon activities (Undheim & Drugli, 2012), focus on relationship quality with peers and adults (Gunnar & Donzella, 2002), and shorter hours whenever possible (Drugli et al., 2018) could help to regulate children's cortisol levels. Caregivers and parents need to give special consideration to children who appear to be less comfortable with childcare.

There is a need for further longitudinal investigation of toddlers' full-day cortisol activity in childcare. Factors that could underlie both cortisol activity and well-being should be studied. Future research should explore and clarify the concept of well-being in childcare and how to enhance it. Cortisol activity in children with low well-being in childcare needs to be investigated. Also, more research is needed to explore the associations between toddlers' temperament and cortisol activity.

As the relation between process quality and toddlers' cortisol activity remains unclear (Drugli et al., 2018; Suhonen et al., 2018; Sumner et al., 2010; Vermeer & Van Ijzendoorn, 2006), researchers should consider incorporating new forms of quality measurements into cortisol studies, such as observations of individual children's experience in childcare (Slot & Bleses, 2018), more fine-grained and comprehensive tools (Guerrero-Rosada et al., 2021; Pianta et al., 2020) or observation of a greater variety of activity and time points in childcare (Slot et al., 2015).

Acknowledgments

The authors thank all the children, parents, and caregivers for their participation in the present study.

Disclosure statement

The authors report no conflicts of interest.

Funding

Thrive by Three and the present study are funded by the Research Council of Norway under the program "BEDREHELSE".

ORCID

Kathrin Nystad http://orcid.org/0000-0003-0844-3642
May Britt Drugli http://orcid.org/0000-0002-2325-8552
Stian Lydersen http://orcid.org/0000-0001-6613-8596
Ratib Lekhal http://orcid.org/0000-0002-3750-4275
Elisabet Solheim Buøen http://orcid.org/0000-0001-7120-4586

References

Ahnert, L., Rickert, H., & Lamb, M. E. (2000). Shared caregiving: Comparisons between home and child-care settings. *Developmental Psychology*, *36*(3), 339–351. https://doi.org/10.1037/0012-1649.36.3.339

Albers, E. M., Beijers, R., Riksen-Walraven, J. M., Sweep, F. C. G. J., & de Weerth, C. (2016). Cortisol levels of infants in center care across the first year of life: Links with quality of care and infant temperament. *Stress (Amsterdam, Netherlands)*, *19*(1), 8–17. https://doi.org/10.3109/10253890.2015.1089230

Berry, D., Blair, C., Ursache, A., Willoughby, M., Garrett-Peters, P., Vernon-Feagans, L., Bratsch-Hines, M., Mills-Koonce, W. R., & Granger, D. A, (2014). Child care and cortisol across early childhood: Context matters. *Developmental Psychology*, *50*(2), 514–525. https://doi.org/10.1037/a0033379

Blair, C., Raver, C. C., Granger, D., Mills-Koonce, R., & Hibel, L, (2011). Allostasis and allostatic load in the context of poverty in early childhood. *Development and Psychopathology*, *23*(3), 845–857. https://doi.org/10.1017/S0954579411000344

Buss, A. H., & Plomin, R. (Eds.). (1984). *EAS temperament survey for children. Temperament: Early developing personality traits*. Lawrence Erlbaum.

Cadima, J., Nata, G., Barros, S., Coelho, V., & Barata, C. (2020). *Literature review on early childhood education and care for children under the age of 3. OECD Education Working Paper No. 243*. OECD Publishing.

Center on the Developing Child. (2021). *Toxic Stress*. Retrieved May 28, from https://developingchild.harvard.edu/science/key-concepts/toxic-stress/

De Schipper, J. C., Tavecchio, L. W. C., Van Ijzendoorn, M. H., & Van Zeijl, J. (2004). Goodness-of-fit in center day care: Relations of temperament, stability, and quality of care with the child's adjustment. *Early Childhood Research Quarterly*, *19*(2), 257–272. https://doi.org/10.1016/j.ecresq.2004.04.004

De Schipper, J. C., Van Ijzendoorn, M. H., & Tavecchio, L. W. C. (2004). Stability in center day care: Relations with children's well-being and problem behavior in day care. *Social Development*, *13*(4), 531–550. https://doi.org/10.1111/j.1467-9507.2004.00282.x

Dettling, A. C., Parker, S. W., Lane, S., Sebanc, A. M., & Gunnar, M. R. (2000). Quality of care and temperament determine changes in cortisol concentrations over the day for young children in childcare. *Psychoneuroendocrinology*, *25*(8), 819–836. https://doi.org/10.1016/S0306-4530(00)00028-7

Dressendörfer, R. A., Kirschbaum, C., Rohde, W., Stahl, F., & Strasburger, C. (1992). Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *The Journal of Steroid Biochemistry and Molecular Biology*, *43*(7), 683–692. https://doi.org/10.1016/0960-0760(92)90294-S

Drugli, M. B., Solheim, E., Lydersen, S., Moe, V., Smith, L., & Berg-Nielsen, T. S. (2018). Elevated cortisol levels in Norwegian toddlers in childcare. *Early Child Development and Care*, *188*(12), 1684–1695. https://doi.org/10.1080/03004430.2016.1278368

Evans, G. W. (2003). A multimethodological analysis of cumulative risk and allostatic load among rural children. *Developmental Psychology*, *39*(5), 924–933. https://doi.org/10.1037/0012-1649.39.5.924

Groeneveld, M. G., Vermeer, H. J., Van Ijzendoorn, M. H., & Linting, M. (2010). Children's wellbeing and cortisol levels in home-based and center-based childcare. *Early Childhood Research Quarterly*, *25*(4), 502–514. https://doi.org/10.1016/j.ecresq.2009.12.004

Guerrero-Rosada, P., Weiland, C., McCormick, M., Hsueh, J., Sachs, J., Snow, C., & Maier, M. (2021). Null relations between CLASS scores and gains in children's language, math, and executive function skills: A replication and extension study. *Early Childhood Research Quarterly*, *54*, 1–12. https://doi.org/10.1016/j.ecresq.2020.07.009

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*, *27*(1-2), 199–220. https://doi.org/10.1016/S0306-4530(01)00045-2

Gunnar, M. R., & Herrera, A. M. (2013). The development of stress reactivity: A neurobiological perspective. In: P.D. Zelazo (Ed.), *The Oxford Handbook of Developmental Psychology: 2: Self and Other*. : Vol *2*. (pp. 45–80). Oxford University Press.

Gunnar, M. R., Kryzer, E., Van Ryzin, M. J., & Phillips, D. A. (2011). The import of the cortisol rise in child care differs as a function of behavioral inhibition. *Developmental Psychology*, *47*(3), 792–803. https://doi.org/10.1037/a0021902

Hanrahan, K., McCarthy, A. M., Kleiber, C., Lutgendorf, S., & Tsalikian, E. (2006). Strategies for salivary cortisol collection and analysis in research with children. *Applied Nursing Research: ANR*, *19*(2), 95–101. https://doi.org/10.1016/j.apnr.2006.02.001

La Paro, K. M., Hamre, B. K., & Pianta, R. C. (2012). *The classroom assessment scoring system. Toddler manual*. Brookes Publishing Co.

Legendre, A. (2003). Environmental features influencing toddlers' bioemotional reactions in day care centers. *Environment and Behavior*, *35*(4), 523–549. https://doi.org/10.1177/0013916503035004005

Lekhal, R., Drugli, M. B., Berg-Nielsen, T. S., & Buøen, E. S. (2020). A model of intervention and implementation of quality building and quality control in childcare centers to strengthen the mental health and development of 1-3-year olds: Protocol for a randomized controlled trial of thrive by three . *JMIR Research Protocols*, *9*(10), e17726 https://doi.org/10.2196/17726

Loman, M. M., & Gunnar, M. R. (2010). Early experience and the development of stress reactivity and regulation in children. *Neurosci Biobehav Rev*, *34*(6), 867–876. https://doi.org/10.1016/j.neubiorev.2009.05.007

Mathiesen, K. S., & Tambs, K. (1999). The EAS temperament questionnaire—factor structure, age trends, reliability, and stability in a Norwegian sample. *Journal of Child Psychology and Psychiatry*, *40*(3), 431–439. https://doi.org/10.1111/1469-7610.00460

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*(1), 2–15. https://doi.org/10.1016/S0018-506X(02)00024-7

Nystad, K., Drugli, M. B., Lydersen, S., Lekhal, R., & Buøen, E. S. (2021). Toddlers' stress during transition to childcare. *European Early Childhood Education Research Journal*, *29*(2), 157–182. https://doi.org/10.1080/1350293X.2021.1895269

Ouellet-Morin, I., Tremblay, R. E., Boivin, M., Meaney, M., Kramer, M., & Cote, S. M. (2010). Diurnal cortisol secretion at home and in child care: A prospective study of 2-year-old toddlers. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *51*(3), 295–303. https://doi.org/10.1111/j.1469-7610.2009.02167.x

Pakarinen, E., Lerkkanen, M. K., Poikkeus, A. M., Kiuru, N., Siekkinen, M., Rasku-Puttonen, H., & Nurmi, J. E. (2010). A validation of the classroom assessment scoring system in Finnish kindergartens. *Early Education & Development*, *21*(1), 95–124. https://doi.org/10.1080/10409280902858764

Phillips, D. A., Fox, N. A., & Gunnar, M. R. (2011). Same place, different experiences: Bringing individual differences to research in child care. *Child Development Perspectives*, *5*(1), 44–49. https://doi.org/10.1111/j.1750-8606.2010.00155.x

Pianta, R. C., Hamre, B. K., & Nguyen, T. (2020). Measuring and improving quality in early care and education. *Early Childhood Research Quarterly*, *51*, 285–287. https://doi.org/10.1016/j.ecresq.2019.10.013

Sajaniemi, N., Suhonen, E., Hotulainen, R., Tormanen, M., Alijoki, A., Nislin, M., & Kontu, E. (2014). Demographic factors, temperament, and the quality of the preschool environment as predictors of daily cortisol changes among Finnish six-year-old children. *European Early Childhood Education Research Journal*, *22*(2), 286–306. https://doi.org/10.1080/1350293X.2013.783303

Salimetrics (2019). Collection Method: SalivaBio Children's Swab (SCS). Retrieved from https://salimetrics.com/wp-content/uploads/2018/02/children-swab-saliva-collection-instructions.pdf

Slot, P. L., & Bleses, D. (2018). Individual children's interactions with teachers, peers, and tasks: The applicability of the inCLASS Pre-K in Danish preschools. *Learning and Individual Differences*, *61*, 68–76. https://doi.org/10.1016/j.lindif.2017.11.003

Slot, P. L., Boom, J., Verhagen, J., & Leseman, P. P. M. (2017). Measurement properties of the CLASS Toddler in ECEC in The Netherlands. *Journal of Applied Developmental Psychology*, *48*, 79–91. https://doi.org/10.1016/j.appdev.2016.11.008

Slot, P. L., Leseman, P. P. M., Verhagen, J., & Mulder, H. (2015). Associations between structural quality aspects and process quality in Dutch early childhood education and care settings. *Early Childhood Research Quarterly*, *33*, 64–76. https://doi.org/10.1016/j.ecresq.2015.06.001

Statistics Norway (2019). Household income, by type of household. Number of households and median (C) 2005–2018. https://www.ssb.

no/97014/median-inntekt-etter-skatt-etter-fylke-og-husholdningstype.kroner
Statistics Norway (2020). August 12). *Befolkningens utdanningsnivå*. https://www.ssb.no/utdanning/statistikker/utniv
Statistics Norway (2021). March 9). *Innvandrere og norskfødte med innvandrerforeldre*. https://www.ssb.no/befolkning/statistikker/innvbef
Stensen, K., Lydersen, S., Stenseng, F., Wallander, J. L., & Drugli, M. B. (2021). Teacher nominations of preschool children at risk for mental health problems: How false is a false positive nomination and what make teachers concerned? *Journal of Psychopathology and Behavioral Assessment*, *43*(3), 646–656. https://doi.org/10.1007/s10862-021-09871-6
Suhonen, E., Sajaniemi, N. K., Alijoki, A., & Nislin, M. A. (2018). Children's biological givens, stress responses, language and cognitive abilities and family background after entering kindergarten in toddlerhood. *Early Child Development and Care*, *188*(3), 345–358. https://doi.org/10.1080/03004430.2016.1218157
Sumner, M. M., Bernard, K., & Dozier, M. (2010). Young children's full-day patterns of cortisol production on child care days. *Archives of Pediatrics & Adolescent Medicine*, *164*(6), 567–571. https://doi.org/10.1001/archpediatrics.2010.85
Tribble, R. C., Dmitrieva, J., Watamura, S. E., & LeBourgeois, M. K. (2015). The cortisol awakening response (CAR) in toddlers: Nap-dependent effects on the diurnal secretory pattern. *Psychoneuroendocrinology*, *60*, 46–56. https://doi.org/10.1016/j.psyneuen.2015.05.009
Undheim, A. M., & Drugli, M. B. (2012). Perspective of parents and caregivers on the influence of full-time day-care attendance on young children. *Early Child Development and Care*, *182*(2), 233–247. https://doi.org/10.1080/03004430.2011.553678
Van Ijzendoorn, M. H., Tavecchio, L. W. C., Stams, G.-J., Verhoeven, M., & Reiling, E. (1998). Attunement between parents and professional caregivers: A comparison of childrearing attitudes in different child-care settings. *Journal of Marriage and the Family*, *60*(3), 771–781. https://doi.org/10.2307/353545
Van Trijp, C. P. J., Lekhal, R., Drugli, M. B., Rydland, V., & Solheim, E. B. (2021). Validation of the Leiden Inventory for the Child's Well-being in Daycare (LICW-D) Questionnaire in Norwegian ECEC centers. *Frontiers in Psychology*, *12*, https://doi.org/10.3389/fpsyg.2021.767137
Vermeer, H. J., & Groeneveld, M. G. (2017). Children's physiological responses to childcare. *Current Opinion in Psychology*, *15*, 201–206. https://doi.org/10.1016/j.copsyc.2017.03.006
Vermeer, H. J., & Van Ijzendoorn, M. H. (2006). Children's elevated cortisol levels at daycare: A review and meta-analysis. *Early Childhood Research Quarterly*, *21*(3), 390–401. https://doi.org/10.1016/j.ecresq.2006.07.004
Watamura, S., Donzella, B., Alwin, J., & Gunnar, M. R. (2003). Morning-to-afternoon increases in cortisol concentrations for infants and toddlers at child care: Age differences and behavioral correlates . *Child Development*, *74*(4), 1006–1020. https://doi.org/10.1111/1467-8624.00583
Watamura, S., Donzella, B., Kertes, D., Gunnar, M., & Watamura, S. (2004). Developmental changes in baseline cortisol activity in early childhood: Relations with napping and effortful control. *Developmental Psychobiology*, *45*(3), 125–133. https://doi.org/10.1002/dev.20026
Watamura, S. E., Coe, C. L., Laudenslager, M. L., & Robertson, S. S. (2010). Child care setting affects salivary cortisol and antibody secretion in young children. *Psychoneuroendocrinology*, *35*(8), 1156–1166. https://doi.org/10.1016/j.psyneuen.2010.02.001

ISBN 978-82-326-7346-9 (printed ver.)
ISBN 978-82-326-7345-2 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (online ver.)

NTNU

Norwegian University of
Science and Technology