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Physical growth, mental development and neuro-endocrine functioning in Portuguese institutionalized children: A longitudinal study

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Resumo

O presente estudo teve como principal objectivo descrever e compreender o crescimento físico, funcionamento neuro-endócrino e o desenvolvimento mental de crianças colocadas em instituições Portuguesas, ao longo da sua permanência na instituição e desde o momento de admissão. Pretendia-se também compreender a contribuição de factores individuais da criança e ambientais para os resultados nos domínios do desenvolvimento acima mencionados. Trinta e uma crianças, com idades até aos 30 meses, foram avaliadas no momento de admissão na instituição e em mais três momentos subsequentes. As cuidadoras institucionais também participaram no estudo. O crescimento físico (Direcção-Geral de Saúde, 2005), desenvolvimento mental (Bayley, 2006), e produção diurnal de cortisol (funcionamento neuro-endócrino) foram avaliados em quatro momentos de avaliação ao longo de 8 meses de institucionalização. Os comportamentos sensíveis e cooperantes (Ainsworth, Bell, & Stayton, 1974) dos cuidadores institucionais em interação com a criança foram avaliados, bem como a qualidade do contexto institucional (Silva et al., 2010). As experiências pré-institucionais da criança foram também analisadas. Observaram-se ganhos significativos no peso e perímetro cefálico das crianças ao longo do tempo, mas não no comprimento. Ao longo do tempo foram persistentes os défices moderados ao nível cognitivo, da linguagem e desenvolvimento motor. A produção diurnal de cortisol foi mais elevada no momento da admissão da criança na instituição, diminuiu durante os meses seguintes, tendo tendido a aumentar novamente após os 8 meses de institucionalização. As experiências pré-natais, a idade e a condição da criança no momento de admissão e a qualidade do contexto institucional contribuíram para os resultados desenvolvimentais.

Abstract

The aim of the present study was to describe and understand the physical growth, neuroendocrine functioning and mental development of children placed in Portuguese institutions from the time of admission onwards. It was also our intention to understand the environmental and individual contributors for the outcomes in such developmental domains. Thirty-one children, with ages up to 30 months, were assessed at the time of their admission at the institution and afterwards, in three time point assessments. Institutional caregivers also participated in the study. The physical growth (Direcção-Geral de Saúde, 2005), mental development (Bayley, 2006), and diurnal cortisol production were assessed in four time points throughout 8 months of institutionalization. The sensitive/cooperative behaviors (Ainsworth, Bell, & Stayton, 1974) of the institutional caregiver towards the child were assessed, as well as the quality of the institutional context (Silva et al., 2010). The child pre-institutional life experiences were analyzed. Significant gains were observed in weight and head circumference growth, but no gains occurred in height growth across time. Moderate deficits persisted in cognitive, language and motor development across time. Diurnal cortisol production was higher at the admission at the institution, diminished in the following 5 months but tended to increase again after 8 months of institutionalization. Prenatal experiences, age and the child's status at the admission at the institution, and the quality of the institutional context contributed to the developmental outcomes.

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Abbreviations

Hypothalamic-Pituitary-Adrenal (HPA)

Intelligence Quotient (IQ)

Prenatal alcohol exposure (PAE)

Fetal Alcohol Syndrome (FAS)

Low Birth Weight (LBW)

Sympathetic-Adrenomedullary System (SAM)

Glucocorticoid (GC)

Corticotropin-Releasing Hormone (CRH)

Arginine Vasopressin (AVP)

Adrenocorticotropic Hormone (ACTH)

Paraventricular Nucleus of the hypothalamus (PVN)

Glucocorticoid Receptor (GR)

Stress HypoResponsive Period (SHRP)

Handling (H)

Maternal Separation (MS)

Licking/Grooming (LG)

Arched-Back Nursing (ABN)

Posttraumatic Stress Disorder (PTSD)

Developmental Quotient (DQ)

Early Life Stress (ELS)

Area Under the Curve with respect to ground (AUCg)

Introduction

Institutionalization in childhood has been studied in depth in recent decades as a social problem of paramount importance. There is growing evidence that institutionalization is an experience of global deprivation with highly unfavorable contours for the child development. It has impact on several domains of development in part due to the limited human and physical resources, disproportionate caregiver-child ratios, poor individualization of care, and reduced sensorial and cognitive stimulation. Also, the pre-institutional experiences seem to play a role in the developmental outcomes in institutionalized children, with particular relevance to the prenatal experiences. Nevertheless, there are individual differences in the outcomes, with some authors pointing out to the role of the quality of care provided in institutions, to the length of institutionalization and also due to the age at admission at the institution (van IJzendoorn & Juffer, 2006).

Traditionally, studies with institutionalized children focused on the effects of this rearing experience on physical growth, intellectual functioning, and emotional and behavioral development (see MacLean, 2003). The classic studies had an important role in alerting to the deleterious and pervasive effects of this welfare response, despite their methodological limitations (see MacLean, 2003). The new wave of studies on institutionalization was triggered by the opening of the doors of many Romanian orphanages at the fall of the communist regime in this country. At that time, these studies were much more controlled and, importantly, have been continued with a longitudinal design, through the international adoption of these children. These studies have formed the basis of the reports on the long-term effects of this early deprivation experience.

In recent years, researchers have sought to find, using neurobiological models, an explanation for the effects of early adversity (e.g., maltreatment, institutional rearing) on further development. It is believed that neurobiological models could entail mechanisms through which psychosocial processes operate and by which individual differences may be explained (Gunnar, Fisher, and The Early Experience Stress and Prevention Network, 2006). Most of these studies are strongly supported and oriented by preclinical research, with animal models, on the effects of early adversity on stress physiology and brain development.

The hypothalamic-pituitary-adrenal (HPA) system has been shown to be a critical system fostering both resilience and vulnerability to stress in animals and humans, with considerable evidence suggesting that early adverse experiences likely contribute to stress vulnerability. The dysregulation of the HPA system may affect the production of growth hormones and have an impact on the child's physical growth. In addition, excessive cortisol production may also have neurotoxic effects on the brain, and thus affect cognitive development.

Nevertheless, only a few studies had the opportunity to study children in a longitudinal way throughout the time in the institutional context, and none, to our knowledge, has done it with a baseline assessment corresponding to the child's admission at the institution and marking the transition between familial and institutional context. Most of the investigations have focused on the effects of institutionalization when the children had already been adopted and consequently the period of deprivation was already over, in many cases a long time before the assessment. In Portugal, the effects of institutionalization on child development have been poorly studied, in contrast to the considerable number of institutionalized children. According to the last report from the Portuguese Institute of Social Services in 2009 there were 9 563 children reared in institutions, of which 850 were under three years of age (Instituto da Segurança Social, 2010). Thus, the present study aims to contribute to overcome this lack of knowledge about the development of Portuguese institutionalized children, especially at the physical, mental and physiological domains, in order to improve social policies and practices implemented when caring for these children.

The present dissertation is divided in two parts. Part I consists of a theoretical and empirical review of the literature, firstly focusing on the conceptual models for understanding the effects of early experiences on development, and then on the empirical studies about the effects of prenatal adversity and parental deprivation on physical growth, mental development and neuroendocrine functioning. Following this, the focus is dedicated to the research about the effects of institutionalization on physical growth, mental development, and neuroendocrine functioning. Part II describes an empirical longitudinal study about the effects of institutionalization on physical and mental development, and also on the physiological regulation of stress. The results obtained are presented and discussed.

PART I

CHAPTER 1

PHYSICAL GROWTH, MENTAL DEVELOPMENT AND NEURO-ENDOCRINE FUNCTIONING IN PORTUGUESE INSTITUTIONALIZED CHILDREN: A LONGITUDINAL STUDY.

THEORETICAL AND EMPIRICAL REVIEW

1. CONCEPTUAL MODELS FOR UNDERSTANDING THE EFFECTS OF EARLY EXPERIENCES ON DEVELOPMENT

The research focusing on the role of early environmental experiences on later development has progressed on the basis of several conceptual models. However, herein we will focus on only four of them: critical period models, experience-dependent models, experience-adaptive programming models and life course or cumulative effects models. These four conceptual models are not entirely distinct, and it should be emphasized that they do not constitute theories and do not hold theory-driven predictions, instead they constitute a broad framework for models of early experience.

Critical period models, often termed experience-expectant or sensitive period models, propose the existence of periods in development during which an experience (or its absence) has a higher impact on the neural circuits inherent to a specific behavior or skill (Greenough, Black, & Wallace, 1987; Knudsen, 2004). That is to say that appropriate environmental input, within a narrow timing of exposure (critical period), is needed for normal development to occur. Otherwise, development will likely be affected, with no further reversible effects of ulterior exposure to typical environments. Since individual differences in the outcome within the normal range are not accounted for, it is assumed that all individuals of the same specie will be equally affected, and resilient functioning will not take place, unless an intervention is provided during that sensitive period (O'Connor, 2006). Most of the well-documented examples of critical periods for acquisition of certain behaviors or competencies come from animal literature, namely birdsong (Thorpe, 1963), vision in mammals (Hubel & Wieses, 1970), and imprinting and social attachments in mouse and rhesus monkey (Insel, 1997; Harlow and Suomi, 1970). Critical period models, at least in their restricted form, have not achieved a high relevance on psychological human development research, since "for most characteristics there is often a wide range of response to environment" (Clarke & Clarke, 2000) and always a reminiscence of plasticity throughout development (MacDonald, 1985). Indeed, in the few cases addressing some aspects of human development in which the restricted model was applied (e.g., vision and language; Johnson & Newport, 1989; Maurer, Lewis, Brent, & Levin, 1999) the approach was biological instead of psychological. However, there is strong evidence of psychological and psychiatric phenotypes that operate through biological critical periods (Chess, 1977; Brown, van Os, Driessens, Hoek, & Susser, 2000). For example, some researchers have

found that children with low birth weight and reduced gestational age (indicators of gestational stress) have a higher incidence of emotional problems (Rice, Jones, & Thapar, 2007). Additionally, if the maternal stress occurred late in gestation, a time during which the brain undergoes rapid growth and is more vulnerable to reduction in oxygen and nutrients, these emotional problems are even more striking (O'Connor, Heron, Golding, Beveridge, & Glover, 2002; O'Connor, Heron, Golding, Glover, & The ALSPAC Study Team, 2003). Besides their relevance in supporting the model, these results point to the importance of assuming an underlying biological model when searching for sensitive periods of particular psychological outcomes (O´Connor, 2006). Interestingly, studies with post-institutionalized children also suggested possible sensitive (but not critical) periods after which cognitive and growth recovery is less successful. Studies with Romanian post-institutionalized children have found that improved cognitive and growth outcomes were most evident for the youngest children randomly assigned to foster care (Johnson et al., 2010; Nelson et al, 2007).

In opposition to experience-expectant models and critical periods of development, some researchers advocate a continual plasticity across the lifespan, that led Kagan (1984 in Sroufe, Coffino, & Carlson, 2010, p. 36) to argue that "life was like a tape recorder with the record button always on, such that new experiences would write over and replace earlier experiences". In this sense, the emphasis of *experience-dependent models* is not on a specific time window in which a particular experience occurs, but rather on the "individual adaptation to specific and possibly unique aspects of the individual organism's environment", with individual differences and resilience being expected (Black & Greenough, 1986). Since early psychosocial adversity tends to be followed by later disadvantage or deprivation (Quinton & Rutter, 1988; Rutter & Robins, 1990), the persistence of effects depend largely on this *continuum* of adversity, with main effects owed to the current rather than the past experiences (Clarke & Clarke, 1976, 2000). Some researchers argue that this model is not inherently developmental, since its main focus is not on how the developmental stage of the individual interacts with the type of environmental input needed or on the lasting consequences of inadequate input, but in stating that there is a modification at the biological level due to new experiences (O'Connor, 2006). However, experience-dependent processes must be considered as they seem to confer individuals the ability to perpetrate plastic adjustments and adaptation in response to continuously novelty (Marshall & Kenney, 2009).

The concept of *experience-adaptive or developmental programming* is quite different from the former ones, but emerged through the aggregation of some elements from them (Marshall & Kenney, 2009). Drawing on sensitive period models, it postulates that experiences occurring in specific periods of development, and the adjustments arising from these, are persistent through programming mechanisms, believed to "set" the biological system of the organism through adulthood. However, somehow invoking experience-dependent models, programming models consider individual differences of the outcomes, within (and outside) the normal range, according to their early personal environmental experiences (O´Connor, 2006). More precisely, programming models highlight the adaptations that individuals have to operate in response to the environment during the sensitive period in order to be well adapted, but pose less emphasis on the plasticity across the lifespan which is characteristic of *experience-dependent models* (Marhsall & Kenney, 2009). Indeed, the influence of the early environmental exposure is long-lasting inasmuch it defines each individual' "set point", dependent on the timing of the input, which will be responsible for later individual differences in subsequent response to stress, for example (O'Connor, 2006). The concept of *experience adaptive-programming* has been applied to distinct focus of human research, contrasting with the prevailing animal investigations on critical periods research. However, programming models have also implied completely biological mechanisms (O'Connor, 2006). We could assume that its origins rely on the "Barker hypothesis" of fetal origins of adult disease. Indeed, Barker has proposed that "Coronary heart disease, Type 2 diabetes, stroke and hypertension originate in developmental plasticity, in response to early undernutrition" (Barker et al., 1993). Because the prenatal programming was for low nutrition, the capacity of the body to metabolize glucose was adapted to early life environment, being unable to adjust to the normal/enriched diets taken later in life (Barker, 1997; O'Brien, Wheeler, & Barker, 1999). In fact, this model highlights the importance of the match between the individual and the ever changing environment as the crucial issue (O'Connor, 2006). More recently, the study of developmental programming has extended its focus to the role of maternal psychological influences, such as maternal anxiety and stress, in the programming of biological systems of the fetus related to stress, such as HPA axis (Seckl & Meaney, 2004; Talge, Neal, Glover, & The Early Stress, Translational Research and Preventive Science Network, 2007; Weinstock, 2008). Importantly, recent data documents the effects of postnatal rearing experiences in the programming of HPA

axis and its relationship with subsequent developmental outcomes (Meaney, Szyf, & Seckl, 2007). Also, there have been sporadic attempts to find psychological mechanisms programmed by early experiences, but these have failed to show any evidence of it. In 2004, Rutter, O'Connor and the ERA study team failed to found an experience-adaptive programming effect for intelligence quotient (IQ) in children adopted from very deprived orphanages in Romania. However, regarding disinhibited attachment, they accepted the explanation of a programming effect by orphanage experiences, but not mediated by cognitive development, as they previously hypothesized.

Another reference that should be emphasized is the *life course or cumulative effects models*. Here, individual differences are placed to the fore and it is highlighted that development comprises complex, bidirectional flows of effects throughout hierarchically distributed systems and subsystems. These models are more devoted to understand developmental trajectories and outcomes in light of the balance between both individual's risk and protective experiences accumulated over time (O'Connor, 2006). In doing so, there is concern in explaining individual differences in later adjustment by examining their pathways or trajectories, and how the exposure to risk or protective experiences shaped their developmental trajectories. Of note is the fact that the importance attributed to early experiences, rely on the nature and significance of subsequent experiences. In fact, cumulative effects models propose that further development is determined by the cumulative history of interactions between the child and his/her environment, rather than by the early experiences solely (Sroufe, et al., 2010). However, it is unquestionable that the early years are worth attention since they demand adaptations from the individual, which are present afterwards in subsequent transactions (Sroufe et al., 2010). In this sense, life course models place individuals in the complex, dynamic and transactional relation between them and their internal and external contexts, more than the other models do (O'Connor, 2006). In this perspective, being early exposed to risk does not imply being destined for disorder. Individuals may diverge from their pathways of origin due to subsequent experiences. (Sroufe, Egeland, Carlson & Collins, 2005). The link between early risk and later negative outcomes is more probabilistic than deterministic, which is different from sensitive period or programming models discussed above (O'Connor, 2006). Although, it does not mean that early experiences are almost erased by subsequent experiences as is hypothesized by experience-dependent models, but they can rather be transformed (Sroufe et al.,

2010). It is also assumed that it is not just one risk factor but a complex of factors throughout development that act combined to predict later outcomes. So, for instance, the presence of several risk factors, in the context of child maltreatment, at any level of the transactions may imply a much greater likelihood of later maladjustment and psychopathological outcomes (Cicchetti & Toth, 1995). Central for these models are the concepts of multifinality and equifinality. The first one means that individuals may have the same initial pathway and exposure to the same risk but diverge to diverse developmental outcomes. On the other hand, equifinality refers to the existence of multiple pathways converging to the same outcomes. That is to say that individuals may begin and traverse different pathways but end up in a similar place (O'Connor, 2006; Sroufe et al., 2005). Similarly to sensitive period and developmental programming models, there is concern about the developmental stage at which the exposure to risk or protective experiences occur, but also the consequences for the next stage that can derive from having or not succeeded in previous developmental tasks as in experience-dependent models (O'Connor, 2006).

These models provide different contributions to the study of the impact of early adverse experiences, but all are equally important. Our view is that a combination of all of them may help to understand the effects of early deprivation.

In studying these effects in humans, researchers have been focusing mainly on the prenatal experiences and on the quality of early caregiving environment (O'Connor, 2006). Both may constitute risk factors for negative developmental outcomes. Additionally, researchers have also become interested in biological mechanisms that might underlie the protracted adverse effects of early experiences. According to developmental programming models, early experiences may program hormonal systems by defining their set points of activation that may persist throughout life. Biological systems under study may be impacted by the quality of the pre and postnatal care, and their altered functioning may also act as a risk factor for the functioning of other human systems. For instance, increased secretion of cortisol by the HPA axis in response to psychosocial stressors may inhibit the production of growth hormones and growth factors by the growth axis and thus impact on child's physical growth (Albanese et al., 1994; Cianfarani, Geremia, Scott, & Germani, 2002). Furthermore, when analyzing the developmental effects of early risk factors, and according with the life course models, it was not one single risk factor limited in time and isolated in nature that produces the

outcomes. Instead, several risk factors must be considered, as well as their cumulative impact on child development. Additionally, risk factors may have a differential impact in different individuals and may act combined with protective factors that must be accounted for the outcomes, as they may weaken the deleterious effects of risk experienced (Luthar, Cicchetti, & Becker, 2000). Importantly, a broader approach integrating the mutual influences of individual variables as well as variables from the multiple environmental contexts where the child is integrated must be considered when looking for the balance between effects of risk and protective factors (see Bronfenbrenner, 1979). This broader view is particularly interesting given the examples of individuals displaying adaptive functioning and development despite adversity, which has been named resilience (Luthar et al., 2000). Given that these individuals are able to maintain normative development even after experiencing early adversity, it is of utmost importance to extend our view and seek for the multi-level processes or mechanisms that may be protecting and potentiating their positive developmental trajectories. In this broader view on the balance between risk and protective factors of adaptive development in adverse situations, it should not be forgotten the developmental timing when adversity occurred, since its effects may vary depending on the child's developmental phase. With this in mind, we will now review the effects of adversity during prenatal life and deprivation from the expectable levels of parental care on physical growth, mental development and regulation of the neuroendocrine stress system.

2. THE IMPACT OF EARLY ADVERSITY ON DEVELOPMENT

2.1. INTRODUCTION

Early life experiences are seen in developmental psychology as primary events that have a profound impact on later human development. The nature and severity of this impact seems to be somehow dependent on the degree and developmental timing of the infant's exposure. There are certain periods in early life, called sensitive periods, during which experiences have a more significant effect on the neural circuits of specific behavior or skill. These are seen as windows of opportunity during which some experiences provide the foundation to the development of certain skills or competencies (Fox & Rutter, 2010). On the other hand, they are also developmental windows during

which organisms are more vulnerable to the enduring effects of certain environmental adverse events or stressors. Structures and systems that seem to be more vulnerable at any given developmental timing are those that are undergoing rapid maturational change (Talge et al., 2007). Although human genetic background provides a cardinal basis for early child development, it must be viewed as a framework upon which several environmental experiences influence future structure and function (Fox, Levitt, & Nelson, 2010). The dynamic interactions between genes and experiences early in life shape the developing brain and set the foundations of brain architecture (Friederici, 2006; Horn, 2004; Katz & Shatz, 1996; Shonkoff, 2010). Therefore, each one of the individual's perceptual, cognitive, and emotional competencies is set up upon the framework provided by early experiences (Fox et al., 2010).

Recent studies suggest that the brain is able to adapt and operate changes throughout the lifespan, despite the decline with age in its capacity for change (Fox et al., 2010; Shonkoff, 2010). In fact, the brain is most flexible and/or "plastic" in earlier ages, when it is more able to accommodate a broad range of experiences and interactions (Shonkoff, 2010). Accordingly, the foundation of brain architecture is based on the development in early ages, and the influence of the environment in basic cognitive processes is much greater at this period of time (Fox et al., 2010). Additionally, similar environmental conditions can lead to different emotional and cognitive experiences, depending upon the child's age (Hensch, 2005; Hess, 1973; Knudsen, 2004). The basic brain architecture is built over time, starting before birth and extending into adulthood. Early experiences have an impact on the quality of that architecture by defining either a prosperous or a fragile foundation for ulterior development. The ability of organization and functioning of the human brain depends on an extraordinary set and sequence of pre and postnatal environmental experiences, which influence the genome. The brain is vulnerable to conditions of extreme deprivation occurring during critical periods of human development, which can lead to lasting damage of the neuro-regulatory systems, with adverse neurobehavioral consequences as an outcome (Shonkoff, 2010).

Early adversity resulting in excessive or prolonged stress can be toxic to the developing brain. When a child experiences extreme poverty or severe maltreatment during prolonged or intensive periods, without the support of an adult caregiver, stress can become toxic and disruptive to the developing brain circuits. Prolonged periods of

stress during early life can also have a cumulative cost on the child's learning abilities as well as on physical and mental health. In this sense, the degree of the adversity experienced in early life seem to be a predictor of the severity of the developmental difficulties showed later on (Shonkoff, 2010). Studies in the field, have been pointing to the fact that exposure to early adversity may contribute to trigger or exacerbate physical and/or psychological disturbances, through mechanisms of developmental programming (Schlotz & Phillips, 2009). A possible way to overcome these effects seems to be through the establishment of sensitive and responsive relationships with caregivers.

The effects of prenatal events and maternal well-being during pregnancy have been intensively studied in the last years with increasing evidence that adverse experiences during prenatal life may also have long-lasting deleterious effects on child development. During the 50s, several studies have warned that complications during pregnancy and delivery, and even the early care environment, may contribute to disturbances in mental health and behavioral problems later on. In the years that followed, these ideas were somehow abandoned because it was considered that the experiments at all ages were influential (Rutter, O'Connor, & The ERA study team, 2004). However, since the 90's researchers began to argue again that the early experiences may have permanent and important effects on development and that these could be magnified by subsequent events (Schlotz & Phillips, 2009). Currently, several studies have been demonstrating robust associations between measures of the quality of the prenatal care, such as birth weight, and the risk of cardiovascular and metabolic diseases in the future (Barker et al., 1993; Barker, 1997). It has become clear that the fetus responds to environmental conditions and one of the consequences are persistent changes both in the structure and physiology of the offspring (Schlotz & Phillips, 2009). Thus, prenatal and neonatal outcomes are often markers for future health, and this has encouraged many researchers to go into the nature of fetal programming. Fetal programming or developmental plasticity refers to the physiological adaptation of the fetus to the characteristics of the fetal environment wherein it is developing. These adjustments may affect the set points of physiological systems that are undergoing rapid development and if these set points are not suited for the postnatal environment the offspring may prove vulnerable to the emergence of health problems later on (Talge et al., 2007).

A comprehensive study on the effects of prenatal adversity demands to also pay attention to the child's postnatal environment, since for the most part dysfunctional and adverse prenatal care are followed by high levels of postnatal risk or stress often in the form of child's maltreatment and neglect (Thompson et al., 1994).

The next section aims to review findings from research on the specific impact of the early adverse experiences on physical growth and mental development, during the pre and postnatal periods. The role of the prenatal care will be analyzed as well as the effects of maltreatment and/or neglect in the postnatal period.

2.2. THE EFFECTS OF EARLY ADVERSITY ON PHYSICAL GROWTH AND MENTAL DEVELOPMENT

2.2.1 EFFECTS OF PRENATAL ADVERSITY AND PARENTAL DEPRIVATION

A considerable body of evidence supports the idea that adverse events experienced during human prenatal development may have enduring effects on postnatal development (Schlotz & Phillips, 2009; Talge et al., 2007). Prenatal adversity can lead to changes in brain architecture, whose specific effects are consistent with the stage of brain maturation and the severity of the insult. Some studies have pointed to the mediating role of the brain development to the later child functioning (Schlotz, Jones, Godfrey, & Phillips, 2008), suggesting that the long-term consequences of adversity may be attributable to the redefinition of several hormonal systems that exert control over child's growth and development. In this respect, it seems to be of particular relevance the hormonal systems that mediate the stress response, especially the HPA system, which will be the focus of our attention in the next section.

Perhaps the best examples to illustrate the enduring effects of adversity during fetal development comes from studies that link exposure to teratogens such as nicotine, alcohol and cocaine with poor postnatal outcomes in physical, cognitive and social domains of development (O'Connor et al., 2003; Talge et al., 2007). But there is also evidence that sub-nutrition and maternal psychosocial stress during pregnancy have an impact on fetal growth and cognitive, social and emotional development later on (e.g., Glover, 2011; Van den Bergh, Mulder, Mennes, & Glover, 2005). These factors may affect fetal environment through alterations in the oxygen and nutrition supply to the

fetus due to the placental vaso-constrictive action of stress hormones or toxins like nicotin, or directly through the transference of maternal glucocorticoids (stress hormones) or other circulating messengers across the placenta (Matthews and Phillips, 2006; Sarkar, Bergman, O'Connor, & Glover, 2008; Wadhwa, 2005). It is clear that differences in fetal growth outcomes are influenced by different adverse prenatal environmental factors (Schlotz & Phillips, 2009).

Smoking during pregnancy can cause both to the mother and the child a myriad of complications ranging from intrauterine growth retardation, abortion, premature birth, low birth weight, sudden death of the newborn, to deficits in child's physical growth (Bada et al., 2005; Knopik, 2009; Knopik et al., 2005; Kyrklund-Blomber, Granath, & Cnattingius, 2005). Thus, cigarette smoking has been associated with behavioral problems in infancy and childhood (Linnet et al., 2005; Milberger, Biederman, Faraone, Chen, & Jones, 1996, 1997), with delays in cognitive, motor and language development (Cornelius, Ryan, Day, Goldschmidt, & Willford, 2001; Fried, O'Connell, & Watkinson, 1992; Fried, Watkinson, & Gray, 1992), as well as with low birth weight (Gilman, Gardener, & Buka, 2008). Concerning the effects on mental development, results are not so consistent. Some studies did not find the expected negative association between smoking and child's cognitive functioning (Kafouri et al., 2009; Gilman et al, 2008), while other authors emphasized the contribution of other variables, such as maternal education and IQ, to the linking between smoking and cognitive functioning. These variables must be considered and controlled for as they attenuate the relation between smoking and cognitive delays later on (Batty, Der & Deary, 2006)

Also, exposure to drugs may lead to specific structural and functional changes in the brain. Exposure to cocaine can lead to permanent changes in limbic cortical structures, which are rich in dopamine that is known for its relevance in the regulation of attention. In this respect, some authors developed a model based on the hypothesis that cocaine exerts a negative impact on neuro-regulatory mechanisms, leading to disruption of behavioral regulation, particularly in attention, emotions and fine and gross motor skills (Stanwood & Levitt, 2001). Other authors, guided by the patterns of changes described above (Stanwood & Levitt, 2001), have identified a significant association between drug exposure and developmental deficits in terms of language, attention and motor functioning early in life. Despite that, some contradictory findings

exist in respect to the link with cognitive deficits. Frank, Klass, Earls, and Eisenberg (2002) did not find an association between being exposed to cocaine early in life with cognitive development at 6, 12 and 24 months. On the other hand, Arendt et al (2004) reported that children exposed to drugs in early prenatal life tended to perform worse than non-exposed peers in cognitive and motor tasks. As the abuse of cocaine is commonly accompanied with abuse of other drugs, as well as maternal medical and socioeconomic constraints that may also impact on cognitive development, these factors should also be considered and controlled when studying these associations (Lester, Boukydis, & Twomey, 2000).

Prenatal alcohol exposure (PAE) also poses increased risk for behavioral problems, cognitive delays and stress reactivity (Haley, Handmaker, & Lowe, 2006; Sood et al., 2001; Streissguth, Barr, Sampson, 1990). Alcohol consumed by the mother crosses the placenta and thus the fetus is exposed to the same concentrations of alcohol found in maternal blood. However, fetal exposure is greater due to his slower metabolism and clearance, since the fetal liver does not have an effective system for the metabolism of alcohol yet (Freire, Machado, Melo, & Melo 2005). Exposure to alcohol during pregnancy exerts a direct toxic effect on cells, leading to cell death in some circumstances. The presence of alcohol may prevent the transport of amino acids, hinder the flow of blood in the placenta, and therefore cause hypoxia and adversely affect hormonal regulation systems that control maturation and migration of nerve cells (Michaelis & Michaelis, 1994, in Streissguth & Connor, 2001). Alcohol is probably the best studied teratogen and specific damage seem to depend on variables such as (a) the amount of alcohol consumed by the mother, (b) timing of the exposure and (c) the conditions of exposure. Moreover, and no less important, are the social conditions to which the child is exposed in the postnatal period that may contribute to developmental changes (Gunnar & Kertes, 2005). Alcohol excessive consumption during pregnancy can result in fetal alcohol syndrome (FAS) that is considered an irreversible condition. The diagnosis of FAS involves three main features identified in the child's face, growth and brain. The facial features involve a specific pattern of craniofacial anomalies (e.g., small palpebral fissures, nasal filter long, and narrow upper lip), including usually convergent strabismus (Streissguth & Connor, 2001). Growth characteristics include weight and/or height delays in prenatal and postnatal periods (Coles et al., 1991; Jacobson et al., 1994: Sood et al., 2001), as well as diminished growth velocity up until

three years old (Day et al., 1991). Regarding neurocognitive development, it is marked by brain damage or dysfunction, as is the case of microcephaly, cognitive delays, fine and gross motor difficulties, hyperactivity and attention deficit. These effects do not tend to mitigate with age as happens with the effects on physical growth (Streissguth & Connor, 2001). Other authors reported deficits in receptive and expressive language in children diagnosed with FAS (Becker, Warr-Leeper, & Leeper, 1990, in Garcia, Rossi, & Giachetti, 2007), as well as delays in cognitive (Streissguth, Barr, Bookstein, Sampson, & Olson 1999) and motor development (Streissguth et al., 1999).

Maternal nutrition during pregnancy is another key contributor to the brain development (Georgieff, 2007). Thus, under-nutrition during pregnancy is associated with fetal growth restriction and, therefore, low birth weight (Rao & Georgieff, 2001). The protein-energy malnutrition is particularly important since it can cause slow intrauterine fetal growth, which may be associated with cognitive deficits later (Gunnar & Kertes, 2005). Deficiencies in micronutrients (e.g., iron, zinc, iodine) during pregnancy may have a negative impact on brain growth and result in long-term consequences in behavior, cognitive and motor development (Colombo et al., 2004; Gale et al., 2008; Hibbeln et al., 2007). For instance, Hibbeln et al. (2007) found that children whose mothers had greater deprivation of omega 3 fatty acids during pregnancy tended to have an increased risk of showing worst performance in motor and language tasks.

There is also another kind of detrimental conditions that could come about during pregnancy and impact on fetal development and later in life: prenatal stress. Prenatal stress could result from the mother's exposure to traumatic events, psychopathology or psychiatric symptomatology, daily hassles and/or negative life events. Anxious and depressive symptomatologies are not rare conditions during pregnancy, instead they occur frequently, more in late pregnancy than in the postpartum period (Talge et al., 2007). Several studies have been suggesting that prenatal stress is associated with some neonatal outcomes, particularly with both a reduction in gestational age and smaller size at birth, which are, *per se*, risk factors for poor developmental outcomes in cognition and social functioning (Wadhwa, 2005; Wadhwa, Sandman, & Garite, 2001; Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993). For instance, in an epidemiological study that measured maternal perception of negative life events between 24 and 29 week of gestation, the authors found that this was related

to increased risk of preterm delivery (Dole, Savitz, Siega-Riz, McMahon, & Buekens, 2003). Also mood disorder symptoms assessed through maternal self-report at 26 weeks of gestation, with emphasis in anxious and depressive symptoms, predicted both premature delivery and low birth weight (LBW) of the newborn (Copper et al., 1996). High levels of anxious or depressive symptoms during pregnancy (assessed by questionnaire) also proved to be related with emotional problems in the offspring (O'Connor et al., 2002; Van der Berg & Marcoen, 2004). In a similar way, a number of studies that used birth weight as a reliable index of the intrauterine growth and quality of the prenatal environment presented a link between LBW and an increased risk of emotional problems later on (Hack et al., 2004; Schlotz & Phillips, 2009; Wiles, Peters, Leon, & Lewis, 2005). Despite this, the quality of maternal care in the early days of life can help to alleviate the effects of stress during pregnancy (Kaplan, Evans, & Monk, 2008). A secure attachment relationship seems to be able to weaken the association between exposure to cortisol in utero and deficits in cognitive development later on (Bergman, Sarkar, & Glover, 2010). Also, the child's genetic background may contribute to the variability in the child's vulnerability to prenatal stress. The fact that children were not equally affected by the prenatal stress led researchers to point to GXE (GeneXEnvironment) interactions as possible explanations of the different outcomes (e.g., Glover, 2011).

Preterm delivery and low birth weight have been seen both as a consequence of adversity during gestation and also as a risk to later developmental outcomes (Gunnar & Kertes, 2005). Prematurity either due to low gestational age (< 37 weeks) or very low gestational age (<= 32 weeks), even if not involving significant brain damage may have an adverse impact on a variety of developmental outcomes (Keltikangas-Jarvinen et al., 2007). Prematurity can also be accompanied by low birth weight (LBW < 2500 grams) or very low birth weight (VLBW <= 1500 grams). Size at birth, mainly birth weight, has been used as one of the principle proxy-measures to reflect the quality of the fetal environment in human studies. Only few studies have used measurements of height and head circumference at birth since it is not clear how much they add to the measurement of birth weight. Despite their repeated use, it is widely accepted that these are crude measurements that represent only a summary measure of the success of fetal and infant development (Schlotz & Phillips, 2009). Size at birth follows a trajectory of growth that is established at a very early stage of development, and the maintenance of the fetus in

this trajectory is dependent on the capacity of the maternal placenta to provide sufficient nutrients and oxygen to the fetus. Genetic factors contribute only with a small part to the regulation of birth weight (Schlotz & Phillips, 2009). Some studies have been revealing differences in the cognitive and language development between pre-term babies born weighing less than 1500 grams and full term babies, with the latter ones performing significantly better than the former ones (Rose & Feldman, 2000). Beckwith and Rodning (1991) emphasize that these cognitive delays tend to be attenuated during school age. Low birth weight may also be influential to the child's later physical growth. Some results showed that low birth weight infants have, at 24 months of age, lower weight, height and head circumference than children who were born with normal weight (Eickmann, Lira, & Lima, 2006). Also, low birth weight children were delayed in mental and motor development in comparison to children who were born with normal weight. Nevertheless, environmental conditions accounted more for these mental and motor outcomes than low birth weight, which led researchers to argue that postnatal care may contribute to overcome the effects of adversity during prenatal life. Postnatal care quality is of relevance for an optimal physical, mental, and neurobiological development. For the normative development to proceed, the child must be integrated at least in an "*average expectable environment*" (Hartman, 1958), that provides adequate health and nutritional care, sensorial stimulation, maternal responsive and sensitive care and opportunities of social interactions. When the environment does not provide the *expectable* care, normative development may be compromised (Cicchetti & Valentino, 2006). This is obviously the case when the child is under conditions of neglect and maltreatment. Child maltreatment may be viewed as the most glaring failure of the expectable environment to adequately provide care, in which a "toxic relational environment" entails few opportunities for normal development and poses serious risk for maladaptation throughout development (Cicchetti & Lynch, 1995; Cicchetti & Toth, 2005). Defining child maltreatment has not been an easy and consensual task among researchers and other professionals who study maltreatment, however there is considerable agreement regarding the four major categories that have been operationalized and distinguished: "1) sexual abuse refers to sexual contact or attempted sexual contact between a caregiver or other responsible adult and a child, for the purposes of the caregiver's sexual gratification or financial benefit; 2) physical abuse refers to injuries that have been inflicted on a child by non-accidental means; 3) neglect refers to failure to provide minimum standards of care as well as adequate supervision;

4) emotional maltreatment refers to persistent and extreme thwarting of a child's basic emotional needs" (Barnett, Manly, & Cicchetti, 1993). Childhood maltreatment is a multifaceted phenomena (Glaser, 2000). It could differ not only in the four types described, but also in severity and duration of the maltreatment behaviors. In addition, different types of maltreatment often coexist, and very often they are not an isolated event in time (Barnett, et al., 1993). According to Cicchetti (2002, p.1416) "Child maltreatment exerts deleterious effects on cognitive, social, emotional, representational and linguistic development, as well as disrupts the development of emotion regulation, secure attachment relationships, an autonomous and integrated self-system, effective peer relations, and the successful adaptation in school". Alongside these developmental domains, maltreatment may also impact in health and physical growth, resulting in *psychosocial growth failure* or *psychosocial dwarfism*. During the last century several studies contributed to the evolution and establishment of the *psychosocial growth failure* as a well-recognized syndrome associated with children' psychosocial deprivation. This is a syndrome characterized by poor growth rates that tended to be manifested by children deprived from the expectable levels of care, which are neglected/abused within their caregiving contexts, whether they are in institutions or within the family settings (Blizzard, 1990; Johnson, 2000a, 2000b). Some authors reported deficits in cognitive development in abused children, and also language delays, as well as other behavioral problems (Strathearn, Gray, O'Callaghan, & Wood, 2001; Vondra, Barnett, & Cicchetti, 1990). The emergence of these deficits/problems seems to be associated with the number of adverse events experienced by the child. However, there is some heterogeneity in the developmental outcomes that may be related to factors such as (a) the severity, chronicity and timing of exposure to maltreatment, (b) the existence of a more positive relationship with other caregiver, and (c) personality. These last two factors may reveal as protective factors (Luthar, 2006).

So far, the protracted effects of pre and postnatal adversity on child development, especially in their physical and cognitive development, have been reviewed. However, little has been exposed on the mechanisms through which these effects can be prolonged and sustained over time. One of the mechanisms that have been increasingly studied is fetal and developmental programming of the hypothalamic-pituitary-adrenocortical (HPA) system that mediates the animal and human stress responses. This hormonal system may be programmed in pre and early postnatal life,

and become permanently dysregulated in their functioning as a consequence of the experienced adversity. The HPA axis can be affected itself by early adversity but can also affect the functioning of other systems. Glucocorticoids, the end product of the HPA axis, are potent steroid hormones that may have toxic effects on brain when they are too elevated and consequently impact on child's cognitive functioning. In addition, these hormones may also interfere with the release of the growth hormone (GH) and therefore have deleterious impact on the child's physical growth. Thus, given its importance as a mediator of the child's stress response and as conveying effects on other levels of child functioning, it seems important at this point to focus on the HPA functioning and on how adversity impacts on it.

2.3. THE EFFECTS OF EARLY ADVERSITY ON STRESS REACTIVITY AND REGULATION

2.3.1. THE HPA SYSTEM

Stressors (actual or perceived) that threaten well being activate a coordinated physiological response involving components of autonomic, neuroendocrine, metabolic and immune systems. However, stress responses in mammals are mainly orchestrated by both sympathetic-adrenomedullary system (SAM) and hypothalamic-pituitary-adrenocortical system (HPA). These two systems are narrowly interrelated as they control each other's activity, and regulation of both takes place at the level of the hypothalamus, where autonomic and endocrine functions are integrated with behavior (de Kloet, Vreugdenhil, Oitzl, & Joels, 1998; Palkovits, 1987). However, for the purpose of this dissertation, we will only focus on HPA axis, a system that exerts slower and more persistent actions than SAM.

Stressors trigger a cascade of events (the stress response) that culminate in elevations of glucocorticoid (GC) hormones (Figure 1).

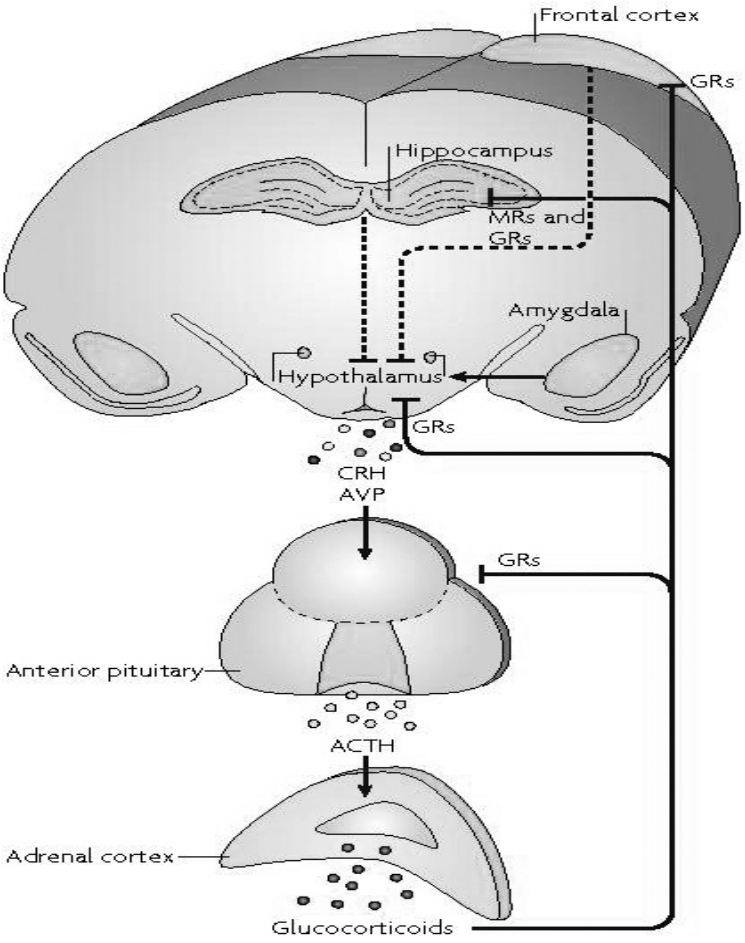

Figure 1: The stress system (Lupien, McEwen, Gunnar, & Heim, 2009).

Such cascade begins with the release of corticotropin-releasing hormone (CRH) along with its cosecretagogue arginine vasopressin (AVP) secreted by neurons in the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN). Both CRH and AVP interact and activate their receptors on corticotropic cells of the anterior pituitary gland, where the adrenocorticotropic hormone (ACTH) is secreted. ACTH circulates in blood and is recognized by receptors on the adrenal cortex, leading to the synthesis and release of GCs (cortisol in primates and corticosterone in rodents) (Johnson et al., 1992a; Gunnar & Quevedo, 2007). GCs then stimulate glucose from energy supplies and enhance cardiovascular tone, among other pervasive effects. This forward loop strengthens the body to anticipate and respond adaptively to a stressor, and is followed by negative feedback loops. Feedback loops, at first mediated by the PVN of the hypothalamus and pituitary corticotroph cells, restore basal levels of activity through

glucocorticoid receptors (GR) suppression of the HPA axis (de Kloet et al., 1998). In general, there is a positive relation between the number of receptors and efficacy of negative feedback (Sapolsky, Krey, & McEwen, 1984). Being lipophilic hormones, GCs easily pass through the blood-brain barrier (Zarrow et al., 1970). Although the brain is a major target of GCs (Bohus et al. 1982), these hormones are released into the general circulation and bind to receptors in target tissues, expressed throughout the body and the brain (de Kloet, 1991). Largely, these glucocorticoid-receptor complexes then act as transcription factors regulating the expression of genes that contain GC-responsive regions. Induced changes in these transcription factors can alter in a lasting way gene expression and change the inherited biological program (Meaney & Szyf, 2005). Thus, the action of GCs has potentially long-lasting effects on the functioning of various brain regions that regulate their release, namely hypothalamus, pituitary, hippocampus, and the frontal cortex (Gunnar & Vazquez, 2006; Lupien et al., 2009; Sapolsky, Romero, & Munck, 2000). Accordingly, cortisol exerts effects on various central neural processes mainly implicated in cognition, memory, and emotion (Cicchetti & Valentino, 2006).

In conclusion, the activation of the HPA axis produces elevations in GCs, which are potent steroid hormones that act by changing internal dynamics in order to promote adaptation and restore homeostasis (Gunnar & Vazquez, 2006). This is a basic mechanism of adaptation to change that supports health and viability, through permissive and suppressive effects of GCs. If GR-mediated effects are acute, brief and controlled, they can be protective to emotion and health; in contrast, when there is prolonged or too frequent activation of the system and lack of control, it can lead to a chronic activation of the system and the suppressive effects could threaten health (e.g., by suppressing the immune system) and increase the risk of stress-related disorders (de Kloet et al., 1998; McEwen, 2000a; Sapolsky et al, 2000). According to Gunnar and Quevedo (2007) this is one of the processes that remain unexplained, i.e., the reason why the activation of GRs, mainly occupied during stress responses, results in such deleterious effects. A plausible argument is that this suppressive or reactive mode mediated by GRs is demanded to terminate stress-induced HPA activation (allostasis mechanism) and, by that, restore homeostasis (Sapolsky et al., 2000). However, when there is prolonged occupation of GRs and the suppressive effects are operating for extensive periods, they lead to more costs than benefits to the body (Gunnar, 2000; Gunnar & Quevedo, 2007). These costs refer to the wear and tear of the organism that

has been termed "allostatic load or overload". Allostatic overload could result from either demanding allostasis response too often or from inefficient operation and management of the allostasis response systems (e.g., fail to shut off the response after the stressful situation is over; fail to respond adequately to the initial challenge, leading to overreaction of other systems) (McEwen, 1998, 2008).

These failures in restoring homeostasis through allostasis could result in up-regulation or down-regulation of the HPA system, at a given level, and consequently in hyper or hyposecretion of the end product cortisol. Historically, since the seminal work of Hans Selye (1936), states of stress have been associated with increased secretion of cortisol by the HPA axis in a way that the terms are often interchangeably used in the literature (Heim, Ehlert, & Hellhammer, 2000). As we have been mentioning, being able to mount a cortisol response to an acute stressor has survival value, and it is believed that brief glucocorticoids elevations could represent an advantage for the individual's ability to respond and cope successfully (Vazquez, 1998). However, repeated and prolonged hyperactivation of the HPA axis may damage the structure and function of the brain (De Bellis, et al., 1999a; Todd, Swarzenski, Rossi, & Visconti, 1995). Interestingly, researchers have been confronted by the fact that low cortisol levels also have deleterious effects on the organism and thus represent risk (Gunnar & Vazquez, 2001; Heim et al., 2000). Hypocortisolism is a fairly recently described phenomenon and researchers are beginning to understand its effects and the mechanisms involved in its etiology. It is accepted that hypocortisolism is not considered a normative response to severe stress, since there is a paradoxical suppression of the HPA axis in individuals who are experiencing chronic stress or conditions of trauma (Cicchetti & Rogosch, 2001a). Hypocortisolism can be characterized by low cortisol, flattened daily rhythm of production, or blunted cortisol secretion in response to a stressor (Heim et al., 2000).

In sum, both hypercortisolism and hypocortisolism can reveal allostatic load, the long-term effect of the physiologic response to stress (McEwen, 1998). While hypercortisolism refers to a protracted cortisol response caused by the inability of the body to terminate an allostatic response, even when the stressor has long gone, in hypocortisolism there is a shortage of cortisol secretion (Cicchetti & Rogosch, 2001a).

Hypocortisolism has been associated with exposure to adverse early life conditions. Experiencing early stress could result in prolonged periods of

hyperactivation of the HPA axis and excessive glucocorticoid release in early life, and this was suggested to support the development of hypocortisolism during childhood and even later in life (Gunnar & Vazquez, 2001). This transition from hypercortisolism to hypocortisolism might occur because of the self-adjusting capacity of the organism. Self-adjusting capacities protect the body against deleterious effects of increased GCs by neutralizing their sustained elevated levels. Regarding the phenomenon of hypocortisolism, a failure seems to occur resulting in an "over-adjustment" (Fries, Hesse, Hellhammer, & Hellhammer, 2005) through mechanisms of down-regulation of GC receptors on diverse levels of the HPA axis, decreased synthesis of CRF, ACTH or cortisol, and/or increased sensitivity to negative feedback inhibition of the neuraxis (Heim et al., 2000; Hellhammer & Wade, 1993). Thus, hypocortisolism is a likely consequence of chronic stress (Fries et al., 2005). Indeed, this perspective is even more consistent if we consider that children living under conditions of maltreatment or institutional neglect tend to show dampened early morning levels of cortisol and also a somewhat flattened daily pattern (for review see Gunnar & Vazquez, 2001). Also infants and preschoolers placed in foster care because of neglect and abusive histories, showed low early morning cortisol levels (Bruce, Fisher, Pears, & Levine, 2009; Dozier et al., 2006). However, this does not necessarily mean that children suffering from chronic stress will definitely produce low levels of cortisol (Gunnar et al., 2006). Instead, according to the results found by Kaufman and colleagues (1997), we can expect to find large cortisol responses to psychosocial stressors in children that remain under conditions of chronic stress due to emotional maltreatment (Kaufman et al., 1997). Also, we might expect, according to findings with post-institutionalized adopted children, that low morning cortisol levels normalize possibly due to the improved care in their adoptive homes (Gunnar, Morison, Chisholm, & Schuder, 2001; Kertes, Gunnar, Madsen, & Long, 2008). After all, hypocortisolism can also have protective effects in specific situations. For instance, low cortisol levels may protect the mother and the fetus against the risk of pre-term delivery (Fries et al., 2005). Since prolonged hypo and hypersecretion of cortisol represent risk for health and development, neither condition is desirable as a chronic state and both should be avoided to self-protect the organism (Sapolsky, 1996).

The human HPA system is relatively mature at birth, however it suffers some developmental changes throughout the first years of life. Despite its maturity, it is under

strong social regulation during these first years, being especially buffered by the child's close relationships with adult caregivers, which may protect the body from harmful hyperactivation of the axis. These developmental changes are next reviewed as well as the role of the caregiving quality in buffering the HPA axis functioning.

2.3.1.1. DEVELOPMENTAL CHANGES AND THE ROLE OF CAREGIVING QUALITY ON STRESS REACTIVITY AND REGULATION

At birth, the newborn HPA system in humans is highly responsive to stressors. In fact, as early as 18 to 20 weeks of gestation, the fetal HPA axis produces stress responses to aversive stimulation (Giannakoulopoulos, Teixeira, Fisk, & Glover, 1999). On the other hand, at birth, the adult-like rhythm of cortisol production is still not established. Instead, newborns show two cortisol peaks 12 hours apart (Sippell, Becker, Versmold, Bidlingmaier, & Knorr, 1978) and by the age of 6 weeks we can find a peak in the early morning and a decrease until the evening (Larson, White, Cochran, Donzella, & Gunnar, 1998). Nevertheless, the circadian rhythm is reliably occurring only by the third month (Price, Close, & Fielding, 1983). By the age of 6 months, the HPA neuraxis is almost mature and until 12 months its responsivity to stressors progressively decreases. This period of hyporesponsivity to stress between 6 and 12 months was hypothesized as the human functional equivalent to a period in rats that has been termed the relative "stress hyporesponsive period" or SHRP, although this assumption is yet debatable (Gunnar & Vazquez, 2006). There is a period in rats, between postnatal days 4 and 14, when it is difficult to elicit a substantial glucocorticoid response to a variety of stressors, and this SHRP seems to emerge to protect the developing brain from the potentially deleterious effects of excessive circulating glucocorticoids (Sapolsky & Meaney, 1986). Because this period of diminished cortisol response in humans is tremendously dependent on the social regulation, especially on the presence and support of the caregiver, it seems that it is much more characterized as a period, in humans, that comes under strong psychosocial or parental buffering of the HPA system (Gunnar, 2003). Indeed, caregiver's sensitivity, responsivity and availability during this period seem to play a crucial role in maintaining this apparent hypo-responsivity to stress. On the other hand, adverse experiences such as neglect or maltreatment may interrupt this stress hypo-responsive period. Thus, child's experiences within their caregiving environment throughout this long period play a significant role

in programming the reactivity and basal circadian rhythm of the HPA axis (Gunnar & Donzella, 2002; Gunnar & Vazquez, 2006).

It remains uncertain for how long this dampening of cortisol response persists, however it seems that the diminished GC response persists throughout the toddler and preschool years until the transition to adolescence. It is believed that the transition to adolescence, marks a period that is hormonally more responsive and also of heightened vulnerability to stressors and increased reactivity of the HPA axis (Spear, 2000). However, it is not yet known the exact timing between childhood and adolescence that marks the transition to a more GC responsive period, and the mechanisms that account for this (Gunnar & Vazquez, 2006). Notwithstanding, when studying the long-lasting effects of early life care on HPA axis reactivity and regulation later in life, we must consider that the link between the two parts may be tenuous until the transition to adolescence (Gunnar & Quevedo, 2008).

Researchers are still not sure about the existence of a human functionally equivalent SHRP, but if it exists, researchers believe that it is maintained by specific caregiving behaviors (Loman, Gunnar, & The Early Experience, Stress and Neurobehavioral Development Center, 2010). This hypothesis derives from research in rodents that suggests that maternal licking and grooming (LG) behaviors and milk into the gut buffers the offspring HPA axis and maintains the SHRP (Rosenfeld, Suchecki, & Levine, 1992). Some authors believe that sensitive and responsive behaviors to infant's signals are the ingredients that most resemble LG in rodents and that these are the two critical dimensions of care in protecting child's HPA axis from adversity (Loman et al., 2010). These ingredients are also main contributors for the emergence of a secure attachment relationship by the end of the child's first year (Ainsworth, Blehar, Waters, & Wall, 1978; Sroufe, 1983). In fact, as is non-human primates, research on the regulation and buffering of the human infant's HPA system has developed supported by the attachment theory principles. Secure attachment relationships proved, both in laboratory and naturalistic experiments, to be a critical buffer in regulating HPA axis activation in stressful situations for the child (Gunnar & Quevedo, 2007). However, in the context of adversity, the care provided is not only inadequate but also the source of stress, and thus can be a source of physiological activation, rather than a buffer. Also,

atypical caregiver's behaviors, such as frightening behaviors and episodes of dissociation may emerge in the context of child's maltreatment and neglect, having potential effects on HPA axis activity (Lyons-Ruth, Easterbrooks, & Cibelli, 1997). This kind of behaviors seems to put infants in risk for developing disorganized attachment relationships, which may per se entail more stress vulnerability (Gunnar & Quevedo, 2007). Disorganized attachment relationships are commonly found in institutionalized children and in high risk samples, and they represent a failure in the attachment system of the child in organizing and balance affect and behavior toward the attachment figure, in stressful events (van Ijzendoorn, Schuengel, & Bakermans-Kranenburg, 1999). Indeed, several studies reported high cortisol levels in response to Ainsworth's Strange Situation Procedure, among children classified as having disorganized (type D) attachment relationships (Hertsgaard, Gunnar, Erickson, & Nachmias, 1995; Spangler & Grossmann, 1997). Acknowledging that HPA axis activity is under social regulation early in life, and quality of care is seriously compromised in maltreatment and depriving contexts it is thought that some of the effects on HPA axis activity found in maltreated and neglected children are mediated by the early care provided both during their pre and postnatal life.

2.3.2. EMPIRICAL RESEARCH ON PRENATAL ADVERSITY AND PARENTAL DEPRIVATION

INTRODUCTION

Exposure to adverse conditions early in life, that could generally be defined as conditions that threaten physical or emotional well-being, may influence the experience-dependent maturation of HPA system, in a way that seems to increase stress reactivity and consequently GR exposure (Gunnar et al., 2001; Lupien et. al., 2000; Seckl & Meaney, 2004; Tarullo & Gunnar, 2006). GCs, the end product of the HPA axis activation, also impact on extrahypothalamic CRH pathways, with some evidence that elevated GCs increase the activity of CRH-producing cells in the central nucleus of the amygdala (Makino, Gold, & Schulkin, 1994). Therefore, elevations in GCs are only one way for early experiences to produce their effects, although it plays a significant role during both prenatal and postnatal development (Gunnar, 2000). Assuming that the activity of stress-sensitive systems are shaped by the interaction early in life between environmental experiences and gene activity, individual differences in the set point of

the HPA axis activity, over the first years of life may occur. However, due to the significant plasticity of the axis, alterations in GCs activity might also occur if caregiving environment changes significantly. Thus, GCs activity and HPA axis set point might reveal the contours of child's life experiences (Gunnar, 2003). Most of the evidence for these assumptions comes from work with animal models of adverse caregiving, in which the study of the effects of stress can develop at the cellular and molecular level. Thus, it is interesting, at this point, to review some of the work on this topic, involving animal studies, before turning out our attention to human studies wherein we will explore the effects of prenatal adversity and parental deprivation on HPA axis activity and regulation.

2.3.2.1. ANIMAL STUDIES

Animal or preclinical studies concerning the effects of early life stress on HPA activity and regulation allow the researchers to manipulate the early environment and by that measure the effects at various levels of the system (including at cortico-limbic structures and pathways) which, due to ethical considerations, is not possible in children. In fact, when searching for neurobiological correlates in children, and subsequent biomarkers of the effects of early life adversity, studies are still largely focused on cortisol measurements. Unable to measure above the adrenal, we must rely on preclinical studies and consider its outstanding contributions, integrated with human developmental knowledge, to inform theory-driven research on HPA system in humans (Gunnar & Vazquez, 2006).

RODENT STUDIES

Handling (H) and maternal separation (MS) are the most commonly used paradigms to study the effect of early environment in animal models. Handling involves removing the pup from the mother and nest, in brief separations (3 to 15 minutes) daily, for the first few weeks of life and results in decreased stress reactivity in adulthood or, in other words, more stress resilience (Levine, 1957; Meaney, Aitken, Sharma, Viau, & Sarrieau, 1989; Viau, Sharma, Plotsky, & Meaney, 1993). Maternal separation implies repeated separation for longer periods (e.g. 3 hours daily, between days 2-14 of life) and

the effects were the opposite of those found with handling, as MS enhances HPA responses to stressors and the pups become more stress vulnerable in adulthood (Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Plotsky & Meaney, 1993). The handling results are not surprising if we take into account that is normal, in the context of the mother-pup interactions, that the mother is absent for 20 to 30 minutes per day (Jans & Woodside, 1990). On the other hand, it seems important to note that, although in rodents the postnatal period is relatively hyporesponsive to stress, being difficult to produce elevations in ACTH and GCs in response to stressors between day 4 and 14 after birth, separation from the dam revealed as one of the most potent stressors able to provoke a response (Levine & Wiener, 1988; Rosenfeld, Suchecki, & Levine, 1992). Researchers have also found that naturally occurring variations within the normal range in the behavior of dams have an impact in the developing neurobiology of stress (Caldji et al., 1998; Liu et al., 1997; Meaney, 2001). These natural variations refer to the amount of licking/grooming (LG) and arched-back nursing (ABN) of the pups by the mothers. Mothers who showed high levels of LG-ABN have pups that, as adults, displayed less behavioral fearfulness in response to novelty, and more modest HPA responses to stress than the offspring of low LG-ABN dams (Caldji et al., 1998; Liu, et al., 1997). We now know that maternal behavior permanently alters the development of HPA reactivity in the offspring through tissue-specific effects on gene expression, particularly during the first week of life. The level of LG-ABN (high or low) of the offspring by dams triggers epigenetic modifications (methylation) at a GR gene promoter, namely in the hippocampus (Weaver et al., 2001). Methylation, leading to silencing, of hippocampal GR gene is thought to be a relevant mechanism through which early experiences affect later stress reactivity and vulnerability in rodent life (Meaney & Zsyf, 2005). Continued deficit in hippocampal GRs may be a potential mechanism for the influence of maternal care in vulnerability to stress-induced effects throughout life.

NONHUMAN PRIMATE STUDIES

Although studies with rodents provide a meaningful framework for better conceptualize the effects of early adverse experiences, we have to keep in mind that the rodent brain is much less mature at birth than the primate brain, which results in a considerable limitation to translate rodent findings. At birth, nonhuman primates have

brain maturation similar to humans and have a long postnatal period of maturation and growth. Interestingly, they form specific attachment relationships to caregivers like humans do (Suomi, 1995) and exhibit similar responses to separation from the attachment figure, displaying intense behavioral distress and increased activity of the HPA and SAM axis (Bowlby, 1968; Hinde & McGinnis, 1977; Levine & Wiener, 1988). Actually, the research on the effects of early experience in nonhuman primates has relied on the theoretical framework of attachment, in which the quality of the mother-infant relationship is viewed as a social regulator (and buffer) of stress responses (Bowlby, 1969; Suomi, 1995). Since they are in close physical contact with their mother early in life, it is possible that even brief separations are stressful enough to provoke behavioral and HPA axis responses (Sanchez, 2006). However, behavioral distress is not always accompanied by similar physiological reactivity, and maternal buffering account for that by preventing physiological stress responses when infant is behaviorally distressed. Indeed, if the infant, during separation periods, can maintain visual or auditor contact with the mother, his/her manifestations of behavioral distress are more evident than if he/she is isolated (no contact at all). But, at the level of HPA axis reactivity, the inverse occurs, with higher physiological responses in the isolation condition (Bayart, Hayashi, Faull, Barchas, & Levine, 1990; Smotherman, Hunt, McGinnis, & Levine, 1979). Studies in which the immediate and long-term effects of brief (30 minutes to 6 hours) but repeated unpredictable mother-infant separations in rhesus monkey were analyzed, researchers found increased cortisol reactivity soon after the separation but a flattened pattern of cortisol production across the day long after the last separation (Sanchez et. al., 2005). The results were in part explained by the alterations (as in rodents) in the relationship between mother and infant due to separation, and by the magnitude of the HPA response to separation stress.

However, the long-term effects of social deprivation on HPA axis in non-human primates are not yet clear, with no evidence yet of alterations in hippocampal GR, as seen in rodents. Nevertheless, in both rodents and nonhuman primates, the maternal caregiving behaviors towards the offspring are thought to impact on the HPA axis. Also, human studies have been guided by this principle, that the quality of the parental care early in life has long-lasting effects on the functioning of the HPA system. We will then review human studies on the effects of prenatal adversity and parental deprivation.

2.3.2.2. HUMAN STUDIES

It has been recognized for many years that children exposed to severe early life experiences such as neglect, abuse, deprivation or trauma are at elevated risk of a number and diverse long-term neuropsychiatric conditions, including drug addiction, depression, post-traumatic stress disorder, and suicide (Cicchetti & Manly, 2001; Dawes et al., 1999; Gilbert et al., 2009; Heim, Owen, Plotsky, & Nemeroff, 1997). However, when it comes to establish a link between early adversity and later disorders, one must be aware that studies in humans lack the imposed experimental manipulation typically seen in animal studies. As a consequence, to study the effects of early adverse experience in humans, one must ground our work on naturally occurring "experiments" and quasi-experimental designs (Levine, 2005). Of course this poses serious questions and difficulties to researchers when trying to translate and integrate basic neuroscience findings from animal models to human research and intervention. For instance, it is almost impossible to study in isolation one single adverse event, since it is rare to find children exposed to only one form of maltreatment. In fact, physical and/or sexual abuse is often accompanied concurrently with neglect, which results in an adverse cumulative effect rather than a main effect of a single adverse experience per se (Barnett, Miller-Perrin, & Perrin, 2005). Also, as Rutter and colleagues (2004, 2007) pointed out, another question in translating animal models to human research is that humans have the capacity to construct and reflect one's own experiences, and the persistent effects of adversity could be determined by individual's cognitive/affective processing of that reflection (Rutter et al., 2004; Rutter, 2007). Besides that, ethical considerations limit our action when trying to study the effects of early adversity on children' neuroendocrine functioning, as it is not allowed to measure HPA functioning above the adrenal level, which is limiting, since its functioning depends on the regulation of the hypothalamus and pituitary, at least. Despite that, it seems that the field is moving towards addressing these translational issues. Indeed, in a recent work by studying postmortem brains of humans who suffered from childhood abuse and committed suicide, researchers found an effect of parental care on the epigenetic regulation of hippocampal GR expression, similar to those found in rodents (McGowan et. al., 2009). Another way to overcome these issues seems to be the study of situations in which a sharp (and measurable) discontinuity occurs in a child's environment, and the adverse period of their lives is circumscribed. One possibility comes from studies with post-

institutionalized children, wherein we can examine whether early adverse experiences had persisting effects later on, when the caregiving environment was enriched (O'Connor et al., 2000; Rutter, et al., 2004). However, we consider of utmost importance to also present some findings regarding the effects of prenatal adversity and parental deprivation since they very often precede and unleash the admission of the child at the institution.

2.3.2.2.1. EFFECTS OF PRENATAL ADVERSITY AND PARENTAL DEPRIVATION ON STRESS REACTIVITY AND REGULATION

Prenatal adversity may have protracting effects on child development through alterations in the functioning of the HPA axis, in addition to autonomic nervous system and insulin-like growth factors (Schlotz & Phillips, 2009). Experiencing adversity during fetal period could result in an elevated bio-behavioral reactivity to stress, with raised activity of both HPA and autonomic nervous systems (Phillips, 2007). It is known that a considerable amount of cortisol is metabolized as it crosses the placenta; however it is suggested that enough passes into the fetal environment with an impact on fetal brain development (Talge et al., 2007). Indeed, a strong correlation was found between maternal plasma and fetal plasma cortisol levels (Gitau, Cameron, Fisk, & Glover, 1998). These systems are believed to be plastic in their set point and can be resetting and programmed or lastingly changed by early life conditions (Phillips, 2002). These can constitute long-term changes, inasmuch they are thought to involve gene methylation of the steroid receptors within the limbic system, and by that implicated in long-term health (Weaver, Cervoni, Diorio, Szyf, & Meaney, 2004). Studies that have used birth weight as a measure of prenatal stress found that people with LBW tended to show increased biological reactivity to stress (Phillips, 2007). For example, in a study where young healthy males were exposed to the Trier Social Stress Test (TSST), which involves a psychological stress eliciting task, their cortisol responses were inversely related with their birth weight (Nilsson, Nyberg, & Ostergren, 2001). Interestingly, maternal stress, depression and anxiety have been linked with enhanced basal levels of cortisol in the offspring at different ages, specifically 6 months (Lyons-Ruth, Wolfe, & Lyubchik, 2000), 5 years (Gutteling, de Weerth, & Buitelaar, 2005), and 10 years (O'Connor et al., 2005). Hyperactivity of the HPA axis is strongly related with depressive and anxiety disorders, which in turn may be explained by a disturbance in the

limbic circuits that could result from alterations during their developmental programming (Ehlert, Gaab, & Heinrichs, 2001). These results seem to corroborate the programming hypothesis; however, it is not clear whether these associations take place independent of the genes shared between mother and offspring (Rice et al., 2007). Another plausible mechanisms that may account for the relationship between fetal adversity and later outcomes has to deal with the *continuum* of adversity that very often characterizes the life history of the offspring. Indeed, the association between poor fetal growth and later depression in early adulthood was strengthened by concurrently being reared under conditions of adversity (Fan & Eaton, 2001).

A considerable amount of research on the effects of child maltreatment indicated atypical physiological regulation of the HPA axis, as well as atypical responses by noradrenergic, dopaminergic and serotonergic systems. Among other elicited responses, such experiences may impose overwhelming stress to the organism, which demands an allostasis response from the HPA axis in order to restore homeostasis and support cognitive, emotional and metabolic activity. Thereby, early life stress in the form of child abuse or neglect may affect individual's psychological and biological development (Cicchetti & Rogosch, 2001b).

A great contribution to the study of childhood maltreatment comes from research with adult survivors who were abused or neglected when younger. However, the findings are sometimes difficult to interpret, mainly because many of these studies used inappropriate controls, didn't measure current life stress, nor did account for current psychiatric status (Gunnar & Vazquez, 2006). The results vary mainly depending on the presence/absence of current psychiatric diagnoses and on the type of stressor used to elicit a HPA axis response (psychological *versus* pharmacological stressor) (Tarullo & Gunnar, 2006). Psychological challenges are situations with potential to elicit a stress response by the individual, such as public speaking. On the other hand, pharmacological challenge consists of an exogenous administration of HPA axis hormones such as CRH or ACTH at a given level of the axis, allowing researchers to localize the functional change in response to the stressor (hyperactivation or hypoactivation of the axis at a given level) (Tarullo & Gunnar, 2006). Subsequently to early maltreatment, many adult survivors developed Posttraumatic Stress Disorder (PTSD) and/or depression (which often coexist), but both of these psychiatric diagnoses imply in itself alterations of the HPA axis functioning, regardless of the occurrence of early life maltreatment. Some

evidence suggests a hyperactivity of CRH at hypothalamic and extra-hypothalamic sites, both in PTSD and depression (Heim, Plotsky, & Nemeroff, 2004). In response to this excess of CRH drive, through counter-regulatory mechanisms, the pituitary becomes down-regulated and a blunted ACTH response is developed (Heim et al., 2004). However, the sensitivity to negative feedback loops is very different in PTSD and depression. An increased negative feedback regulation characterizes PTSD (Young, Haskett, Murphy-Weinberg, Watson, & Akil, 1991), which results in cortisol hypo-secretion. On the other hand, depression is associated with diminished negative feedback (Yehuda, 2000), and as a consequence hyper-secretion of cortisol. Although the confounding variables (e.g., lack of measurement of current life stress and/or current psychiatric status) were not controlled in many studies, there is some consistency in that the adult survivors of childhood maltreatment tend to respond to psychological stressors with increased ACTH secretion. However, when adults are free from psychopathology despite the maltreatment history (this means resilience), it seems that the adrenal is somehow unresponsive to the elevated levels of ACTH and responds with normal levels of cortisol production (Tarullo & Gunnar, 2006). On the other hand, the concurrent presence of PTSD or depression seem to deprive the organism from this counter-regulatory mechanism and adult survivors will present either elevated ACTH or cortisol responses. This hyperreactivity to psychological stressors demands activation of cortico-limbic pathways and thereby suggests that these non-resilient adult survivors may be even more hyper-responding to stressors at the cortico-limbic level than are normal controls with PTSD or depression (Gunnar & Quevedo, 2007).

Although relevant, studies with adult survivors of childhood maltreatment bear many difficulties when used to compare to child maltreatment findings. Indeed, unlike studies with maltreated children, most of the adult studies focused on stress reactivity instead of basal levels of HPA functioning. Moreover, most of them used pharmacological challenges, which are rarely used in early ages due to ethical issues. There is also need to consider developmental changes in HPA axis during childhood and especially in the pubertal transition to adulthood, that may account for the results (Gunnar & Quevedo, 2007; Tarullo & Gunnar, 2006).

Accordingly, when studying the impact of maltreatment during childhood, we must be aware that the neural system is still developing. Given that, we should expect similar or even higher complexity in interpreting children data, since the effects of

developmental changes and maltreatment effects can be difficult to disentangle (Cicchetti & Tucker, 1994). As we mentioned, most of the research in childhood maltreatment focused on basal activity rather than reactivity of the HPA axis. In fact, few studies have measured cortisol reactivity to pharmacological and psychological stressors. One of the studies that did this, using pharmacological challenges, found blunted ACTH response to CRH stimulation among sexually abused girls, though cortisol levels were normal (De Bellis et al., 1994). However, in another study using CRH challenge depressed, abused children responded with enhanced, rather than blunted, ACTH secretion and normal cortisol response, but only if they were experiencing ongoing adversity in family environments (Kaufman et al., 1997). This finding suggests that the environment where the child is currently living has the potential to buffer and protect HPA axis from dysregulation, emphasizing the plasticity of the neuroendocrine system (Cicchetti & Valentino, 2006). With regard to psychological challenges, among the quasi-absent studies, Hart and colleagues (1995) reported that maltreated preschoolers showed lower cortisol levels on the days they got so behaviorally upset and aggressive that they need to be physical restrained by their teachers, comparing to the days when their behavior was more controlled (Hart, Gunnar, & Cicchetti, 1995). Turning now to the studies where basal activity was measured, we continue to find great diversity on the outcomes. However, as it was the case in adults, the current psychiatric status of the child could account for the heterogeneity of the results. According to the dimensions of internalizing versus externalizing disorders, internalizing disorders in children and adolescents have been associated with elevated basal activity of the HPA axis (Goodyer, Herbert, & Althan, 1998; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001). When combined with child maltreatment, researchers have also observed elevated basal levels compared to controls. For instance, De Bellis and colleagues (1999b) observed that for maltreated children with PTSD, 24-h urinary free cortisol (UFC) concentrations were elevated comparing to normal controls (De Bellis et al., 1999b). Also Carrion and colleagues (2002) found elevated salivary GC levels over the day in maltreated children with PTSD relative to normal controls. Regarding results with depressed, maltreated children, similar findings were reported in two day camp studies, wherein a significant rise in GC levels over the day were noted (Carrion et al., 2002). This was not true for normal controls and non-depressed maltreated children (Hart, Gunnar, & Cicchetti, 1996; Kaufman, 1991). In an attempt to isolate the effects of maltreatment from the main

effects of internalizing problems, Cicchetti and Rogosch (2001a,b) measured salivary GC levels over 5 days in maltreated children with internalizing disorders and in non-maltreated children with internalizing disorders. Higher average morning (9 am) and afternoon (4 pm) levels were observed for the maltreated children. On the contrary, maltreated children with PTSD didn't differ from non-maltreated children with clinical levels of anxiety in terms of 24-h UFC concentrations (De Bellis et al., 1999b). So far, it is not yet certain that maltreatment interacts with internalizing disorders to hyperactivate the HPA axis above the hormone levels that are expressed in internalizing problems themselves (Tarullo & Gunnar, 2006).

In turn, children with externalizing disorders in the absence of comorbidity, with anxiety and other internalizing problems, tend to show low levels of cortisol comparing to normal controls (McBurnett, Lahey, Rathouz, & Loeber, 2000; van Goozen, Matthys, Cohen-Kettenis, Buittelaar, & van Engeland, 2000). Accordingly, if child maltreatment enhances GC levels, when combined with externalizing disorders, the resulting basal activity may not be significantly higher than levels of cortisol from normal controls (Gunnar & Vazquez, 2006). Indeed, Cicchetti and Rogosch (2001b), when comparing maltreated children with clinical levels of externalizing problems with non-maltreated children with externalizing disorders, found higher basal cortisol levels for the maltreated group, but only for boys. However, GC levels akin to the levels of normal controls, in other words, they are not elevated above the levels of non-disordered, non-maltreated children (Cicchetti & Rogosch, 2001b).

Beyond the need to measure children's current life stress and consider their psychiatric status to interpret the effects of maltreatment, we must also consider that many physical and sexual maltreated children are also exposed to considerable levels of neglect that may add and *per se* impact on HPA functioning. Neglect should be approached apart from studies of maltreatment since it reflects insufficient or depriving care, which not necessarily means that it is a traumatic or frightening experience for the child (Gunnar & Vazquez, 2006). However, because neglect often co-occurs with other type of maltreatment it has proved almost impossible to isolate its effects (Gunnar & Vazquez, 2006). In an attempt to do it, Cicchetti and Rogosch (2001b) found that, in their studies with summer camp maltreated participants, only 8% of them were exposed exclusively to neglect, and that was not associated with alterations in cortisol levels. Overall, studies show that neglect had a minor effect on basal cortisol levels (Gunnar &

Vazquez, 2001, 2006). Notwithstanding, a great contribution for this topic comes from studies reporting the effects of institutionalization on the HPA axis functioning, largely accepted as reflecting the effects of severe neglect. These studies will be reviewed in the next section.

3. THE EFFECTS OF INSTITUTIONALIZATION ON DEVELOPMENT

3.1. INTRODUCTION

Institutionalization of children has been long adopted as a welfare response to children whose biological parents were unable to care properly. At the same time, there is another welfare response that implies the foster care of these children by families who are paid for. In a study covering 15 European Union countries, including Portugal, authors sought to identify the reasons for the admission at the institutions of children under three years of age. They presented data showing that in Europe *institutionalization* is imposed, on average, to 11 in each 10,000 children, under 3 years of age, with variations between countries (Browne, 2004, in Johnson, Browne, & Hamilton-Giachritsis, 2006). Most of the children were withdrawn from their families and placed in institutions due to parental negligence or abuse (69%), some due to abandonment (4%) or specialized educational needs (4%), and a considerable part due to socio-economic problems (e.g., arrested parents).

The research dedicated to studying the long-term effects of early orphanage rearing on child development is now quite exhaustive and substantial (for review see MacLean, 2003). During the 40s and 50s researchers as John Bowlby (1951), William Goldfarb (1945), and Rene Spitz (1946) have made important contributors to the field. Studies conducted in British orphanages sought to identify the effects of institutional care in child's physical, intellectual and language development (Spitz, 1946; Provence and Lipton, 1962). These studies revealed considerable deficits in multiples developmental domains, with children in orphanages revealing lower overall results comparing to children in the community. Additionally, Spitz (1945) reported a progressive deterioration during the first months of institutionalization at the cognitive level of development. These results were coherent with the ones from Goldfarb studies (Goldfarb, 1945) who compared the cognitive and social development of

institutionalized *versus* foster children. Goldfarb (1945) analyzed the development of institutionalized children in to different time points: when they were still in the institution and seven months after being placed in a foster family. In both time points the author noted significant differences between the two groups in cognitive and language development, with the children who had been institutionalized showing worst results below the values of reference.

Given these results, Spitz (1945) argued that the deficits found were due to the lack of stimulation and opportunities of interaction in the institutions, and the fact that they are deprived of maternal care. These results contributed at the time to the rearrangement of the English institutions, so that they become closer to a family context of care. Children were distributed in smaller groups of children with different ages and care was provided by a larger number of caregivers (Tizard and Joseph, 1970).

Later, other authors, such as Barbara Tizard and colleagues (Tizard, Cooperman, Joseph, & Tizard, 1972; Tizard & Hodges, 1978; Tizard & Joseph, 1970; Tizard & Rees, 1974, 1975) conducted several studies to assess the efficacy of the changes made in the English institutions. Tizard and Tizard (1971) examined the quality of institutional care based on the number of interactions established between the child and caregivers, and between the child and other adults and their peers. They also assessed the differences between institutionalized children and their peers living in the community, in terms of the opportunities to engage in social experiences. They concluded that institutionalized children are cared by a higher number of caregivers and, moreover, were exposed to less social experiences outside the institutions. Authors also reported that institutionalized children were delayed in terms of their cognitive development (Tizard & Joseph, 1970; Tizard & Tizard, 1971), although differences between them and community children were moderate (Tizard & Rees, 1974). These results, although more positive, should be understood in light of the quality of the care received in the institutions where the observations were made, since these children were not subject to extreme deprivation as in previous studies (MacLean, 2003).

Tizard and colleagues (Tizard et al., 1972) also presented results of the effects of institutional characteristics in language development. What they found was an association between the way the institution was organized and the amount of verbal child-caregiver interactions established. These interactions were more frequent when the

caregiver had received specific training for their work. With regard to the conversations, they entailed few opportunities for language enrichment, as they were limited to verbal commands. The authors found that children who were institutionalized in the better quality institutions and the older children had average scores above the reference values for language development. Thus, Barbara Tizard and her colleagues attempted to explain these results supporting them on institutional context characteristics. The results were also supported by other studies in showing that the quality of institutional context is critical for child development (e.g., Dennis, 1976 in Van Ijzendoorn & Juffer, 2006).

More recently, the effects of early institutionalization have been increasingly studied since the fall of the Romanian dictator, Nicolae Ceausescu, in December of 1989. Pervasive impairments across several developmental domains have been described. Although some developmental domains are well studied, as physical growth and cognitive functioning (for review see MacLean, 2003), others are not. Specifically regarding neuroendocrine effects of institutional-rearing, much is still unknown.

In general, institutions failed to provide several aspects of an expectable early environment. Almost all institutionalized children do not have access to several of the experience-expectant features that should be present in a typical environment, such as access to a caregiver, adequate nutrition, sensory stimulation (e.g., visual, auditory, tactile), linguistic input, low exposure to stress or providing the "building blocks to cope with stress" (Nelson, 2007, p.16). A general overview of the characteristics of the institutional context will be presented next as well as the specific effects of institutionalization on physical growth, mental development and neuroendocrine functioning.

3.2. AN OVERVIEW OF THE INSTITUTIONAL CONTEXT

Institutional care settings present some variability both within and between countries, but most of them are characterized by being depriving contexts. Taking the example of Romanian orphanages, they are mostly characterized in the literature as providing only food, clothing and shelter needed for survival (Johnson et al., 1992b). Most of them assign an exaggerated number of infants (sometimes around 60) to only one caregiver, who is occupied all the time in completing life-sustaining tasks,

restraining any kind of personal contact. Also, high staff turnover hinders the development of stable relationships. Furthermore, infants spend most of the daytime in their cribs, with few contacts with peers and caregivers because it facilitates caregiver's work. Confined to other tasks, caregivers rarely interact directly with children, and due to that, there are few moments of joy and laugh, and sounds of play or music are almost nonexistent (for review see Johnson, 2000a). Infants are hardly ever fed by the caregivers instead bottles are propped, and when necessary solid food are spooned briskly into a child's mouth. Needless to say that routines are highly regimented, with all daily activities such as sleeping, toileting, and eating occurring for all at the same time, with little consideration for individual needs (for review see Johnson, 2000a). Toys are often inaccessible and infants and toddlers lack opportunities for free play and interaction with older children, since groups of children are segregated by age. The *leagane* (Romanian orphanage for children up to 3 years) failed to provide a nurturing environment as was expected at home, and in contrast is much similar to the sterile environment of a ward (for review see Johnson, 2000a). Additionally, caregivers are often clinically depressed, for the most part because they are burdened with responsibilities at work, most are underpaid, and face life difficulties that are overwhelming. This kind of caregiver's burnout undermines the quality of the interactions between them and children to whom they provide care, turning to be unresponsive to children signals and needs (for review see Johnson, 2000a). Accordingly, cognitive, language, and sensory stimulation is almost lacking (Nelson et al., 2007). The above description of institutional context fits well Romanian orphanages where the deprivation was really severe. Recently van Ijzendoorn and colleagues combined several accounts to better describe what is common amidst considerable heterogeneity in institutional care, as follows (van IJzendoorn, et al., 2011, p. 10).

- "Group sizes tend to be large (typically 9-16 children per ward, although in extreme cases, the number may approach 70). The number of children per caregiver is large (approximately 8:1 to 31:1, although a few institutions have fewer children per caregiver).
- Groups tend to be homogeneous with respect to ages and disability status. Children are periodically "graduated" from one age group to another perhaps as many as two or three times in the first 2 or 3 years of life.

- Caregivers for any single child tend to change constantly because there may be a high staff turnover; caregivers may work long shifts (e.g., 24hr) and be off 3 days; caregivers may get up to 2 months vacation. The result is that child may see anywhere from 50 to 100 different caregivers in the first 19 months of life.
- Other adults tend to come and go in children's lives, including medical and behavioral specialists, prospective adoptive parents, and volunteers who may visit for only a week or a few months.
- Caregivers typically receive little training, and the training they do receive is more focused on health issues than on social interaction. They spend the vast majority of their hours feeding, changing, bathing, cleaning children and the room, and preparing food rather than interacting with the children. Caregivers are invariably female, so children rarely see men.
- When caregivers perform their caregiving duties, it is likely to be in a business-like manner with little warmth, sensitivity, or responsiveness to individual children's emotional needs or exploratory initiatives."

Descriptions above are part of a framework often denominated by researchers in the field as benign neglect.

Because of the heterogeneity of the institutional experiences, Gunnar (2001) developed a hierarchy, with three levels of privation, ordered by whether children's needs in the institutional context are met or not. Basic needs, such as health and nutrition were included as the most basic to be met. It follows children's developmental stimulation at a sensorimotor, cognitive and language level, as well as preoccupation with child-child and adult-child social stimulating interactions. At the top of the hierarchy are the relationship needs, which include forming emotional attachments and/or stable and consistent interactions with a specific person (Gunnar, 2001). Thus, level 1 of privation refers to a global privation, wherein basic needs, stimulation and relationship needs are not met; level 2 deal with meeting nutrition and health needs, but not stimulation and relationship needs; level 3 of the hierarchy refers to institutions providing all support except for the need of consistent and longstanding relationships with a specific caregiver (Gunnar, 2001). Another level of deprivation was suggested to be added to the hierarchy (van IJzendoorn, et al., 2011), namely the kind of institution that provides for stable and consistent caregiving, but depriving children only of a

regular family life. This fourth level of institutional environment may be represented by the SOS Children's Villages, that are organized as a set of family houses with six to eight children in each house, cared by a stable caregiver during the 24h, 7 days per week. This is a very expensive institutional setting, but according to some authors all institutions should pursue this family-like organization in order to provide better care conditions to children (The St. Petersburg-USA Orphanage Research Team, 2008, see Chapter VIII).

The severity and persistence of the deficits that have been reported in institutionalized children seem to depend on a number of factors, such as the quality of care experienced prior to institutionalization, the age of institutional placement, the quality of institutional care (level of deprivation) and the length of institutionalization. The higher the adversity in each of these criteria the greater the negative impact on development. Some authors (e.g., Nelson, 2007) argue that the cumulative exposure to adversity early in life may lead to permanent neurobiological changes causing developmental deficits in several domains. The effects of institutionalization on physical growth, mental development and neuroendocrine functioning will next be reviewed .

3.3. THE EFFECTS OF INSTITUTIONALIZATION ON PHYSICAL GROWTH AND MENTAL DEVELOPMENT

Growth failure is nowadays considered a universal finding in institutionalized children with results from every study reporting moderate to severe delays in physical growth (Johnson et al., 2010; Miller et al., 2009; Rutter, 1998; Smyke et al., 2007; The St. Petersburg-USA Orphanage Research Team, 2005; Van Ijzendoorn, Bakermans-Kranenburg, & Juffer, 2007). Several recent studies reported on growth failure in children reared in institutions. Johnson and his colleagues examined the health condition of 65 Romanian adoptees entering the United States, and identified that most of the children (around 85%) were impaired in terms of physical health at the time of their entry into the adoptive families (Johnson et al., 1992b). In a study with 475 previously institutionalized children from Eastern Europe, authors found that 72% of children were identified by their adoptive families as having low weight at the time of adoption, and 80% were below normal in height (Groze & Ileana, 1996). Consistent with these figures are the results from two studies with internationally adopted Romanian children in

Canada. The study from Morison, Ames and Chisholm (1995) reported that, in terms of weight, 85% of the sample was below the 10th percentile, and 59% was below the 5th percentile. In another study, authors found that within the 16 Romanian adoptees, 50% fell below the 5th percentile for weight and 44% fell below the 5th percentile for height (Benoit, Jocelyn, Moddemann, & Embree, 1996). Additionally, Rutter and the ERA Study Team (1998) found in their sample of 111 children, with a mean age of 6,6 months, previously institutionalized in Romania and adopted into the UK, that 51% were below the 3rd percentile in length, 34% in weight and 38% in head circumference. Although most of the studies focused on Romanian orphans, the detrimental effects due to institutionalization on linear growth were reported worldwide, such as in studies with adoptees from Former Soviet Union (Albers et al., 1997), from China (Johnson & Traister, 1999), or children in Korean orphanages (Kim, Shin, & White-Traut, 2003). In this latter study, the authors randomly assigned 58 institutionalized children in Korean orphanages during the first weeks of life to two different groups: (a) a control group, in which children were subjected exclusively to routine care, and (b) an experimental group, in which children were subjected to 15 minutes of tactile and visual stimulation twice a day during one month. Stimulation was provided in a highly structured manner, with no signals of responsiveness towards the child. The authors concluded that children in the experimental group had gained more weight and height, and had a larger head circumference immediately after the intervention and also at 6 months of age. This study is reminiscent of the study recently reported by Johnson and Gunnar (2011, p. 93) as follows:

"Perhaps the most convincing early demonstration of how an adverse emotional environment affects growth took place after World War II in Germany, when British nutritionist Elsie Widdowson studied 50 children…in two small municipal orphanages…(Widdowson, 1951). A young, cheerful woman who was fond of children cared for one group of children and an older, stern woman who was a strict disciplinarian to all children except for a small group of favorites cared for the second group. During the first 6 months of observation, the children cared for by the younger woman gained weight and height far better than those in the orphanage governed by the strict matron, with the exception of her favorites who did quite well. During the second 6 months, arrangements were made to provide additional rations to one of the orphanages and, concurrent with the improvement in daily calories, the caretakers shifted as well. During the second 6 months, despite receiving additional calories, the

children in the orphanage managed by the stern matron grew poorly. Her favorites, who accompanied her to the other institution, again were the exceptions and gained weight and height better than either of the other two groups in the study. The children previously cared for by the strict matron and now cared for by the cheerful woman during the second 6 months rapidly gained weight and height despite no increase in calories."

These two studies are interesting in that they strongly corroborate the "*psychosocial short stature"* presented by some authors (Blizzard & Bulatovic, 1990; Johnson, 2000a; Johnson, 2000b, Skuse, 1993) according to which children's exposure to a context of social and emotional neglect can lead to growth deficiencies. After removal of the child from the institutional depriving context, there is usually a sharp and immediate recovery (Johnson, 2000a; Johnson, 2000b; Johnson, 2002; Juffer & van IJzendoorn, 2009) that is likely due to an improvement in both nutrition and increasing secretion of growth hormone (Johnson, 2000a), which reinforces the hypothesis that institutional care may exert an adverse impact on the child's neurobiological and physical growth. Indeed, some authors have argued that for every 3 months of institutionalization, children lose approximately one month of linear growth (Johnson, 2000a). The fact that adopted children commonly show a pattern characterized by significantly higher weight than height, as well as a greater distribution of subcutaneous fat than muscle area in child's upper limbs, led researchers to believe that its etiology is in the hyposecretion of the growth hormone and not due to protein-energy malnutrition (Johnson, 2000a). Moreover, studies reveled that the higher the length of time within the orphanage context, the worst the outcomes (Ames, 1997; Johnson et al., 1992b; Rutter & the ERA Study Team, 1998). The fact that many years after adoption children who were institutionalized remain smaller than their peers in the community may be a consequence of long-lasting neuroendocrine changes, due to institutional placement at an early age and stay for a significant period of time of their lives in the institutional context (Johnson, 2000a; van Ijzendoorn & Juffer, 2006).

Pioneer studies on the consequences of extreme deprivation on mental development began more intensively in the 1940s and 1950s and suggested compromised outcomes in intellectual development for children reared in institutions (Spitz, 1945, 1946; Goldfarb, 1945). Focused on intellectual development, Spitz (1945,

1946) noted a drastic decrease in developmental quotients (DQs) of institutionalized children throughout the first months of institutionalization. Spitz (1945, 1946) claimed attention for the progressive deterioration of the infant's DQs even after improvements in the physical conditions of the institution. This fact led the author to argue that children were permanently impaired due to the institutional placement in their first years of life. Also Goldfarb (1945) studied children placed in institutions during their first three years of life, who were then moved to foster care. These children were compared with a group of children reared in foster care since their early infancy. Goldfarb reported that children who were firstly reared in institutions were intellectually delayed comparing to the foster care group, even in adolescence (Goldfarb, 1945). Given this result, he argued that the deleterious effects of institutionalization were not overcome by moving children to more stimulating and loving environments. Despite their importance and contribution to the field, these studies were highly criticized due to the methodological limitations and lack of details provided regarding either the institutional conditions or the measurements used to assess children (see MacLean, 2003). Later, Barbara Tizard and her colleagues countered these results by demonstrating that children who were reared in institutions during their first 2 years in United Kingdom were not destined for delayed development (Tizard, 1977). Children reared in these institutions showed throughout time only slightly lower language and IQ scores comparing to their peers from the community. However, Tizard's sample had not experienced the extreme deprivation of other samples as the one from Goldfarb's study, and this may be one potent explanation for their positive findings.

Recently, studies with Romanian orphans documented the deleterious effect of severe deprivation on cognitive development. While still institutionalized, 25 Romanian orphans aged between 23 to 50 months, were assessed by Kaler and Freeman (1994) in terms of their cognitive functioning using the Bayley Scales of Infant Development (Bayley, 1969). They revealed that all children were functioning below their age level, and that 20 of the 25 children of the sample were severely delayed. Notably, deficits were not related to length of time in the orphanage, age at entrance, Apgar scores, or birth weight. In another study, Carlson and Earls (1997) studied a group of Romanian institutionalized infants aged between 2 and 9 months that, in terms of cognitive development assessed using the Bayley, scored well below the norms for their age. Several researchers had the opportunity to study the intellectual development of

previously institutionalized Romanian children, and these studies may well be represented by two main research programs, The English and Romanian Adoptees (ERA) study and The Bucharest Early Intervention Project (BEIP).

The ERA study team focused his longitudinal research on the development of Romanian orphans internationally adopted into the United Kingdom (e.g., Beckett et al., 2006; O'Connor et al., 2000a; Rutter & The ERA Study Team, 1998; Rutter, Kreppner, & O'Connor, 2001; Rutter, O'Connor, & The ERA Study Team, 2004). Retrospective assessments were used to measure the children's abilities at the time of adoption, which occurred at up to 42 months of age. These assessments revealed significant delays in children's cognitive development at the time of entry into the UK. However, most children benefited from being adopted with significant improvements in cognitive development, even the ones most severely delayed at the time of adoption (Beckett et al., 2006). Nevertheless, there was a persistent degree of cognitive impairment for a considerable proportion of Romanian adoptees, at 4, 6, and 11 years assessments (Beckett et al., 2006; O'Connor et al., 2000a; Rutter, O'Connor, & The ERA Study Team, 2004). Interestingly, the time spent in the institution prior to adoption was a significant indicator of the cognitive gains showed in adoption. Specifically, children adopted before the age of 6 months had significantly higher gains in the cognitive domain than children adopted after this age point (Beckett et al., 2006; Rutter, O'Connor, & The ERA Study Team, 2004). Although several studies (O'Connor et al., 2000a; Rutter & The ERA Study Team, 1998) reported that length of institutionalization was the best predictor of children's IQ when adopted, a meta-analysis conducted by van IJzendoorn and Juffer (2006), where more than 270 studies were analysed, suggested that catch-up for IQ after adoption is almost complete, but a longer period of institutionalization does not seem to reduce the IQ catch-up.

In a similar way, the BEIP study also concerns Romanian orphans who were institutionalized in their early years; however, instead of being adopted, children were placed in foster care families as an intervention. This research is also particular, especially in regard to its design. Researchers were able to conduct a randomized trial, where children living in institutions were randomly assigned to one of two conditions: (1) being placed at foster care that was provided by high-quality foster families trained and supervised by the BEIP infrastructure; (2) remain institutionalized in the Bucharest orphanages receiving the usual care. Before randomization, when assessed with Bayley

Scales of Infant Development, institutionalized children displayed moderate cognitive delays as a group, when compared to home-reared Romanian children. After assignment to foster care, children revealed higher scores of intellectual functioning at 42 and 54 months of age, compared to the children that remained institutionalized (Nelson et al., 2007). Furthermore, being assigned to foster care between the ages of 7 to 24 months, was an indicator of higher gains in cognitive outcomes, compared to the children placed in foster families after 24 months of age. Notwithstanding, the mean scores on Bayley were always lower in the group assigned to foster care, compared to the community sample, even for the ones placed earlier in foster care. This data is consistent with the results from the studies conducted by the ERA study team in revealing a dose-response to the length of institutionalization in terms of cognitive catch-up, but also in detecting some heterogeneity in the outcomes with persistent delays for some individuals (Beckett et al., 2006; Nelson et al., 2007; Rutter, O'Connor, & The ERA Study Team, 2004).

3.4. THE EFFECTS OF INSTITUTIONALIZATION ON STRESS REACTIVITY AND REGULATION

When studies of institutionalized children began to assess neuroendocrine functioning, researchers, based on preclinical studies, expected to find increased levels of basal cortisol production or high cortisol reactivity to stressors, or even both patterns. Unexpectedly, the results have shown the opposite (Gunnar and Vazquez, 2001). Carlson and Earls (1997) were the first to examine cortisol levels among orphanage-reared children. They studied a group of 2-year-old children, residing in a Romanian orphanage (*leagane*) and compared them to an age-paired sample of family-reared children. They found that the family-reared children showed the normal daily pattern of cortisol production, with a peak in the morning, followed by a continuous decrease across the day. Contrarily, the *leagane* children failed to follow an expected daytime rhythm, showing low levels in the morning and no systematic decrease over the course of the day. These results suggested that orphanage experience disturbs the rhythm of the HPA axis, although it does not necessarily lead to absolute increases in cortisol levels as was originally expected. Similar results were found in a study with Russian institutionalized children (Kroupina, Gunnar, & Johnson, 1997 in Tarullo & Gunnar, 2006), and with children who were neglected by the biological families, soon after being transferred to a foster family (Dozier et al., 2006). Interestingly, these findings resemble

the ones with rhesus monkey infants, in which low levels of cortisol and no significant variation across the day were associated with disruptions in maternal care (Coplan et al., 1996, 1998, 2000; Sánchez et. al., 2005). However, in a small study with Ukrainian institutionalized children, Dobrova-Krol and colleagues (2008) found an expected daytime variation among institution-reared children, regardless of whether they were chronically or temporarily stunted (Dobrova-Krol, van IJzendoorn, Bakermans-Kranenburg, Cyr, & Juffer, 2008). Importantly, the institutions where this study took place were characterized by the second level of institutional privation (according to Gunnar's hierarchy, 2001), which seems to differ from the former studies, and may account for the differences observed. Furthermore, multiple studies have also been conducted once these previously institutionalized children were living with their adoptive family, in order to study the effects of institutionalization on the HPA axis functioning later on. The emerging picture from these studies suggests that removal from early life stress (ELS) conditions to an enriched and supportive environment reverts the flattened pattern of diurnal HPA activity into a typical one. However, there is also some evidence for residual elevations in basal cortisol levels. Gunnar and colleagues (2001) reported that, several years after being internationally adopted, children who had lived for more than 8 months in the orphanage had higher cortisol levels at least at some times during the day, than children who spent only the first 4 months of age in the orphanage (Gunnar et al., 2001). However, the daytime rhythm is fairly normal, with decreases in cortisol from morning to bedtime. Thus, it seems that a longer period under deprivation in the orphanage indicates a more reactive HPA axis. Also, in another study, children who had experienced more severe neglect during this period had higher levels of cortisol in the basal condition after adoption (Fries, Shirtcliff, & Pollak, 2008). Recently, in a study with children adopted from institutions around the world, researchers found slightly higher wakeup levels of cortisol 6-7 years post-adoption, but only for those who were physically stunted at adoption (Kertes et al., 2008). According to this, it seems that being exposed to more adverse early life conditions predicted growth delay, which in turn predicted cortisol levels. These findings suggest that cortisol daytime rhythm may not be permanently affected, even though early experiences in the orphanage, when severe enough, may up-regulate cortisol activity. However, it seems important to note that none of the studies presented above objectively measured the quality of care that is provided in the institutions, which we are certain is an important variable to explain individual differences later in life. In

fact, a recent study (Gunnar, Frenn, Wewerka, & Van Ryzin 2009) conducted with international adopted children aged between 10 to 12 years old, attempted to overcome these issues, by contrasting children later adopted from institutions (severe ELS) and earlier adopted from foster care (moderate ELS) and a non-adopted group (NA) and measured their HPA axis response to a psychological stressor, the Trier Social Stress Test for Children (TSST-C). Surprisingly, they found that children from severe conditions did not differ from the NA group, while moderate ELS was associated with lower cortisol activity. Furthermore, maintaining normal growth despite exposure to adversity seemed to predict diminished levels of cortisol activity (for both groups). The overall results pointed to a lowering effect of moderate ELS and a lack of effect of severe ELS. However, authors also noted that TSTT-C may not fit the age of tested children in terms of eliciting stress reactivity, since only few children from the overall group showed cortisol elevations as a response; additionally, the pre-pubertal status of the children might also confound the results. In fact, in the study of Fries and colleagues (2008) the cortisol reactivity was measured after a 30-min stress-eliciting interaction task between child and mother (or unfamiliar adult), wherein child was regularly engaged in timed and standardized close physical contact (Fries et al., 2008) . The results showed that post-institutionalized children had higher cortisol levels after interacting with their parent than the control group. Indeed, caregiving environment and more specifically the quality of the infant-adult relationship and parental care is thought to be one of the most important moderators of early adversity effects that should be further explored.

Interestingly, recent studies with institutionalized children have been reviving the hypothesis that the pattern of cortisol production could correlate the effects of adversity on physical growth, which may also be extended to the effects on cognitive development since cortisol levels may have toxic effects on brain when they are too elevated. Some literature on this subject will next be presented.

3.5. STRESS CORRELATES OF PHYSICAL GROWTH AND MENTAL DEVELOPMENT

Several authors suggested that the effects of early adversity on the HPA axis regulation might be one of the mechanisms that partly mediates the effects of adversity on physical and psychological development (Gunnar, 2000; Heim, et al., 1997).

Increased activity of the HPA axis, manifested by elevated levels of CRH and cortisol, is suggested to impact on the growth axis, through inhibition of growth hormone and growth factors under conditions of psychosocial deprivation and early neglect (Albanese et al., 1994; Cianfarani et al., 2002). Linear growth delay (height), especially if accompanied by a balanced weight-for-height proportion, may reflect exaggerated drive of HPA hormones on the growth axis. Thus, one can assume that exposure to early severe deprivation, resulting in children's growth delay, could indicate that they may have experienced chronic stress effects on the HPA axis (Kertes et al., 2008). Recent studies with internationally adopted children have found that elevated levels of basal cortisol, several years after being removed from the institutional adverse conditions, may be more likely for children who experience severe stunting in linear growth consistent with "*psychosocial short stature"* (e.g., Kertes et al., 2008; Gunnar et al., 2009). Also, in children who remained institutionalized, Dobrova-Krol and colleagues (2008) have found an association between elevated levels of cortisol and physical growth delays. In addition, extremely low cortisol levels can also be deleterious to the normal GH release since physiological amounts of cortisol are required to support pituitary response to GH releasing hormone (Giustina et al., 1989). Obviously, it is not possible to exclude that malnutrition induced by intestinal parasites, poor nutrition, illness endemic to many orphanages and prenatal growth restriction may also account to growth delays at adoption (Johnson, 2001).

Cognitive deficits in post-institutionalized children have been suggested to arise from the exposure in early life to neurotoxic levels of glucocorticoids that resulted from the chronic activation of the HPA axis (Marshall & Kenney, 2009). This was suggested, for example, by Chugani and colleagues (2001) based on their study using functional neuroimaging with positron emission tomography (PET) with ten children adopted into the USA who were reared in severe deprived orphanages in Romania during their first three years of life (Chugani et a., 2001). The authors proposed that what they found in these ten children in terms of cognitive and behavioral issues, may be linked to the decreased brain glucose metabolism showed in the medial temporal lobe and the inferior temporal cortex. Considering the harmful physiological effects caused by exaggerated levels of glucocorticoids on neural functioning reported on the stress literature, the suggestion made by Chugani and colleagues (2001) that dysfunction in the two brain regions identified by them resulted from adverse effects of chronic stress responses

seems very plausible (Sapolsky et al., 2000). Biological perspectives on the effects of early psychosocial stress and maltreatment were guided by the assumption that early adversity leads to dysregulation of the HPA system and consequently the brain and behavior are also affected (Cicchetti & Walker, 2001). Thus, guided by this perspective, we should expect that the cognitive delays observed in severely deprived children in institutions are partly due to early and protracted HPA axis dysregulation, that leads to persistent sub-optimal levels of cortisol that in turn may have effects on child's cognitive development and functioning (Marshall & Kenney, 2009).

4. CONCLUSION

The early institutionalization of children has been the focus of several studies in recent years, aiming to understand the impact of this kind of deprivation experience, since it is one of the most common welfare response for *orphan* children in many countries. Children placed in institutions and exposed to deprivation of an adequate parental care early in life are at increased risk for the emergence of several pervasive delays that may persist over time if no intervention takes place. The high staff turnover and lack of opportunities to establish one to one interactions with the caregiver contributes greatly to the almost absent individualization of care with a specific and preferred caregiver. These circumstances, along with some others, do not contribute to the *expectable environment of care* within which children would grow successfully. Multiple deficits have been reported in studies comparing institutionalized children with children in the community, with the former ones showing consistently worst outcomes comparing to their peers in the community. Pre and postnatal history of care, prior to institutionalization seem also to contribute to these negative developmental outcomes. However, there is heterogeneity in the outcomes, with some children doing well in these depriving contexts. This led us researchers to seek for protective and risk factors, both within child, in terms of their individual characteristics, and also at the level of care provided by the biological families and the institutional caregivers.

In this respect some researchers sought to identify specific vulnerabilities in the structure of the institutional environment that are implicated in the developmental outcomes, as are the child-caregiver ratios, the dimension of the groups of children, the structure of the caregivers' shifts, but also the quality of the child-caregiver relationship,

in terms of the caregivers' sensitive and responsive behaviors towards the child, as well as the quality of the attachment relationship. The child's neuroendocrine functioning, specifically in terms of the HPA reactivity and regulation, have also been increasingly studied as a possible mechanism of vulnerability to the persistence of these developmental outcomes. Studies with post-institutionalized children, have been allowing researchers to study the effects of institutionalization when children are living in enriched environments, which supposedly contribute to revert the effects of institutionalization. Indeed, data have been showing that the flattened daily pattern of cortisol manifested in institutions is apparently reverted when children are living with the adoptive parents (Gunnar et al., 2001; Kertes et al., 2008). However, more studies in which children are under conditions of stress again (e.g., stressful controlled procedures) are needed in order to clarify if the effects of early life stress may be dormant, or actually overcame.

Our longitudinal study, presented in the next part of this dissertation aims to contribute to the understanding of the child's development during a period of his/her institutionalization, as well as the role of the pre and postnatal experiences previous to institutionalization in his/her developmental trajectory. We also seek to understand the contribution of the quality of the institutional caregiving to the child's outcomes.

PART II

CHAPTER 2

PHYSICAL GROWTH, MENTAL DEVELOPMENT AND NEURO-ENDOCRINE FUNCTIONING IN PORTUGUESE INSTITUTIONALIZED CHILDREN: A LONGITUDINAL STUDY.

AIMS, HYPOTHESIS & METHOD

The present chapter summarizes an original study developed to assess and comprehend the physical, mental and neurobiological development of institutionalized infants, aged between 0 to 30 months, who were admitted at Portuguese institutions between March 2008 and October 2010. Aims, research questions and hypothesis will be next outlined. Subsequently, the design of the study will be described along with the method. Descriptive data relating to participants and institutions will be presented as well as the analyses plan.

1. AIMS

According to the last report on institutionalization from the Portuguese Institute of Social Security (ISS, 2010), in the year of 2009 there were 9 563 institutionally-reared children, of which 7 376 were admitted in the institutions before the year of 2009. Also, according to the figures presented, 57% of the children remained institutionalized for more than two years, and there were only 658 children placed in foster care families. Indeed, this kind of welfare response was observed to be more common in older children, since only 30 children younger than 3 years were integrated in foster care families. Needless to say that, in Portugal, most of the children that were abandoned or withdrawn from their biological families were placed in institutions as a welfare response that is meant to be temporary. However, it is important to note that, for a considerable number of cases - 37% in 2009 - this temporary response exceeded the duration of four years and therefore, for the children under three years of age, roughly 27% remained institutionalized for one year, and 11.8% for about two to three years (ISS, 2010).

According to the extant and international literature on the effects of institutionalization accrued to date, these figures are somehow alarming, and it is worrying that until now there have been scarce attempts in Portugal, especially for those children under three years old, to examine whether this welfare response is adequate for the child's needs. For instance, in the above cited report, the authors aimed to account on mental development and health of the children, by identifying children with behavioral problems, cognitive delay and mental retardation for various age ranges.

Nevertheless, it is still surprising, considering the results from international studies, that none of the institutionalized children under 3 years old were identified has having behavioral problems, and only a residual number were identified within the range from 4 to 5 years. Also, according to this report, very few children are cognitively delayed within the age range from 0 to 5 years.

The study on the effects of early deprivation has received renewed interest since the fall of the communist regime of Ceausescu, when the world became aware of the shocking environmental conditions of severe deprivation in which many abandoned and orphan children were reared in several Romanian orphanages. The situation encountered in these orphanages provided an unfortunate "natural experiment" on the effects of early deprivation on children's development. The contributions of these studies proved to be far-reaching, considering that "by explaining abnormal ontogenetic processes in the social, emotional, cognitive, and linguistic domains, contributions can be made to theories of normal development" (Cicchetti & Wagner, 1990, p. 247). The deleterious effects found as a consequence of institutionalization in Romanian orphanages were alarming and very worrying, especially given that they cover various developmental domains, ranging from cognitive and physical growth delays, going through emotional and behavioral problems, to the alterations in the physiological regulation of stress. This wave of studies on the effects of institutionalization extended to other countries, such as Greece and Ukraine (Vorria et al., 2003; Dobrova-Krol et al., 2008; Dobrova-Krol, van Ijzendoorn, Bakermans-Kranenburg, & Juffer, 2010). In these last countries, the levels of deprivation experienced by children in institutions were not so severe as in Romanian orphanages, and, in our view, the configuration of the institutional environment presented in these countries seems to be the closest to the Portuguese reality. Notwithstanding, the superior quality of care in these institutions did not imped the researchers to found a range of developmental deficits, at the cognitive, physical and socio-emotional levels, and also physiological dysregulation, that need to be carefully assessed and intervened by clinicians, researchers and policy makers (Vorria et al., 2003; Dobrova-Krol et al., 2008; Dobrova-Krol et al., 2010).

Accordingly, it urges that the effects of institutionalization in Portugal are deeply studied and understood. To the best of our knowledge, there is no study so far in Portugal with institutionalized children under 3 years old reporting on physical, mental and neurobiological development. It is our interest and major aim to contribute to a

better understanding on the developmental implications of this rearing experience, and to inform, based on evidence, the opinion and decisions on whether institutionalization is an adequate welfare response to the children aged from 0 to 30 months. In this regard, a longitudinal study was designed and conducted as a part of a broader study conducted in Portuguese institutions that aimed to identify the physical, mental and emotional developmental trajectories of children in institutions and analyze the quality of care provided. Children were assessed first at the admission at the institution, which constitutes their baseline assessment, and afterwards approximately every 3 months. With this baseline assessment we established a control of the change in the caregiving environment.

Within this larger research project design, the present study aims to assess children's transactional relations between their own development and their external proximal and more distal contexts. In this sense, a multilevel approach was delineated to cover individual, relational and contextual variables that may impact on child development. Additionally, one would expect that one of the mechanisms by which early adversity may represent risk for child development is through programming of the HPA axis by early life stress, assuming also an experience-adaptive programming framework, although plasticity may occur.

With this in mind, we aim to characterize children's development and functioning, from the time of admission onwards, at the physical, mental and neuroendocrine level, as well as the impact of the child status at the admission, in particular to the child's neuroendocrine functioning, since that moment could be seen as a stress-eliciting event. Additionally, given that some studies reported that cortisol levels represent stress correlates of physical and mental development, we seek out to add to this knowledge, by correlating cortisol levels with children's physical and mental outcomes (Albanese et al., 1994; Cianfarani et al., 2002; Cicchetti & Walker, 2001; Heim et al.,1997).

Assuming that institutionalization is often preceded by adverse experiences, in prenatal life and/or within the biological family of origin, our study also attempts to capture the risks that children likely experienced during their early life and to understand their contribution to children's developmental status throughout the institutionalization period.

We also seek to understand what are the contributions of the quality of institutional context and relational care for the children's developmental outcomes throughout the institutionalization period. Guided by some translational research, and bearing in mind that maternal care in rats, particularly the behaviors of licking and grooming the puppies, impact on the regulation of the stress responses, we seek to understand whether caregiver's sensitivity toward the child has an equivalent effect in buffering the HPA axis of institutionalized children. Maternal sensitivity has been suggested in the literature as the human equivalent of licking and grooming behavior in rats, although this hypothesis has never been tested in institutionalized children (Loman et al., 2010).

2. RESEARCH QUESTIONS AND HYPOTHESES

Research questions and hypotheses were organized according to the three developmental domains we are interested in examining and to explain whether and how institutionalization experience and previous adversity early in life impact on them. Accordingly, we here start to outline the research questions and hypotheses that refer to physical growth, going then through the research questions and hypothesis regarding mental development, and finally to the ones regarding neuroendocrine functioning and development, specifically cortisol production and regulation.

2.1. PHYSICAL GROWTH

2.1.1. How did physical growth evolve over the time spent in the institution, from the time of admission?

Most of the studies with post-institutionalized (PI) children revealed that, at their initial pediatric visits post-adoption, children consistently showed physical delays, in height, weight and head circumference (Ames, 1997; van Ijzendoorn et al., 2007). Accordingly, we would expect that children in our study, still living in institutions, showed delays in physical growth, that would be generalized for height, weight and head circumference (Benoit et al., 1996; Johnson et al., 1992b; Johnson, 2000a,b; Morison et al., 1995; Rutter & The ERA study team, 1998). Also, studies showed that

the growth deficits are tightly linked to the amount of time the child has spent in an institution. Indeed, according to Johnson (2000a), children lose approximately 1 month of linear growth for every 3 months in institutional care. Thus, we would expect, especially for height, that delays would be as great as the length of institutionalization increase (Ames, 1997; Johnson et al., 1992b; Rutter & the ERA Study Team, 1998). Nevertheless, we wouldn't expect that the severity of the delays in our sample were as high as the ones found by studies with Romanian orphans, since Romanian orphanages were characterized as contexts of severe deprivation, which we believe is not the case in our Portuguese institutions, where deprivation seems to be not so severe and in this sense not so deleterious to child's physical growth.

2.1.2. Was there any effect of age at admission at the institution in the pattern of evolution throughout institutionalization of height, weight, and head circumference?

The quality of care provided in institutions seems to be much less suitable for young children's needs (Castle et al., 1999). Others observed that "for much of the first year of life human infant can do little to produce effects on the environment without the active intervention and mediation of adults. Thus, depriving environments deprive the human infant not only of passive stimulation, but of response-contingent stimulation as well" (Gunnar, 2001, p. 619). Taking this into account, we would expect that children admitted under 12 months of age at the institution, when most of the stimulation that child has the chance to experience is response-independent (Provence & Lipton, 1962), would reveal worse physical growth across time.

2.1.3. What was the etiological role of prenatal risk and birth weight in children's physical growth?

Few studies focusing on the developmental impact of institutionalization had the opportunity to study the children while they were institutionalized, and had much less access to the prenatal conditions prior to institutionalization. Nevertheless, studies as the one conducted by the BEIP group (Johnson et al. 2010) showed that children born with low birth weight (LBW) are at increased risk of growth failure while institutionalized. In this sense, these authors (Johnson et al. 2010) suggested that the assignment of these

children to foster care is much more urgent, arguing that they have unique nutritional needs that are unlikely to be met in institutional contexts, as is also unlikely that institutions are able to provide nutritional interventions that can enhance growth. It is our expectation that a significant association between birth weight and ulterior physical growth is found, and also between the prenatal risk experienced and child's physical growth while institutionalized.

2.1.4. Was physical growth across time associated with child's physical status at the admission?

It is our expectation that, for some children, institutionalization will be beneficial while for others it will not. Being a welfare response, we should expect that institutions, at least, protect children from severe risk factors to which he/she would likely be exposed if they remained with the biological family, and somehow, the new context to where the child is moved, can be positive and allow for some physical gains. To some extent, what we expected to find is similar to what Johnson and colleagues (2010) found when they moved children previously institutionalized to foster families. Children who gained most with this change of contexts were those who were more physically impaired while institutionalized. It is also expected that age at admission at the institution will be related to the evolution of physical growth across time.

2.1.5. Was physical growth across time associated with individual differences in the early familial context, including the severity of the adversity experienced?

Few studies focusing on the developmental impact of institutionalization had the opportunity to study the children while they were institutionalized, and had much less access to the family rearing conditions prior to institutionalization. Nevertheless, and guided by the review from MacLean (2003), we hypothesized that prior conditions and risk that children experienced in the early family context before entering the institution would contribute, in a relevant way, to the outcomes throughout institutionalization. Additionally, we would expect that a picture of growth suppression does exist when infants enter the institution, likely due to neglect and maltreatment experienced in the biological families, as was found for children entering foster care families (King &

Taitz, 1985; Olivan, 2003; Wyatt, Simms, & Horwitz, 1997).

2.1.6. Was physical growth across time associated with the quality of institutional environment and care provided in the institution?

Again, we would expect that not only the prior conditions in the early family context would contribute to the outcomes but also the quality of care experienced in the institution. It is known that children living under stimulation-poor environments show generalized massive physical growth delays (Benoit et al., 1996; Johnson et al., 1992b; Johnson, 2000a,b; Morison et al., 1995; Rutter & The ERA study team, 1998). However, the orphanage context and experience proved to be heterogeneous (Gunnar, 2001), and by that we would expect that this heterogeneity in the quality of care provided would be reflected in a related heterogeneity in the outcomes for physical growth. In the report from BEIP study (Johnson et al., 2010), one of a few that measured caregiving quality in institutionalized children, caregiving quality was only found as a significant predictor of catch-up in height and weight for children assigned to foster care, but not for children who remained institutionalized. We expect to find such an association in our sample of institutionalized children. Indeed, results from an intervention study in Russian Baby Homes showed that a socio-emotional-relationship intervention promoted an effectively improvement in child's physical growth (The St. Petersburg-USA Orphanage Research Team, 2008).

2.1.7. How did caregiver's sensitivity and cooperation associate with the children's physical growth across time?

Although only few studies observed a contribution of the caregiver's interactive characteristics to the child's physical growth, we would expect that a relation between caregiver's sensitive and cooperative behaviors and child's physical growth does exist. Guided by a study conducted after the World War II, in two small orphanages within the British zone of occupation (Widdowson, 1951, in Johnson, 2000a), we would expect that children cared by more sensitive and cooperative caregivers would have better physical growth across time. In this study, two groups of children were cared by two distinct caregivers, "a young, cheerful woman who was fond of children cared for one

group of children and an older, stern woman who was a strict disciplinarian to all children except for a small group of favorites cared for the second group" (Johnson, 2000a, p. 137), and the results indicated that the group cared by the younger caregiver, during the first 6 months, "gained weight and height far better than those in the orphanage governed by the strict matron, with the exception of her favorites who did quite well" (Johnson, 2000a, p. 137). More recently Johnson and colleagues found, although in a sample of previously institutionalized children currently under foster care, a positive association between caregiver's sensitivity and positive regard for the child and their physical growth (Johnson et al., 2010).

2.2. MENTAL DEVELOPMENT

2.2.1. How did mental development evolve over the time spent in the institution, from the time of admission?

Studies with institutionalized children began to reveal permanent impairments for mental development with the pioneering studies of Spitz (1945, 1946) and Goldfarb (1945). Studies with institutionalized children from Romania also revealed a very worrying scenario, since results pointed to cognitive functioning for all children below their age level, and for most of them severe delays were noted (Carlson and Earls, 1997; Kaler and Freeman, 1994). Also, studies with post-institutionalized children indicate that the delays were pervasive across all areas of mental development, and that the time children spent in the institution prior to adoption is a significant predictor of the cognitive functioning at adoption and the gains showed after adoption, rather than the time in the adopted home (Beckett et al., 2006; O'Connor et al., 2000; Rutter, O'Connor, & The ERA Study Team, 2004; Rutter & The ERA Study Team, 1998). These findings led us to expect that delays would be pervasive, impacting on cognitive, language and motor functioning, and would increase as length of institutionalization extends.

However, again, we wouldn't expect that the severity of the delays in our sample are as high as the ones found by studies with Romanian orphans, since they were not reared in contexts characterized by the severe deprivation encountered in Romanian orphanages. In fact, outcomes vary according to the severity of deprivation encountered

in the institution, as is the good example shown by the results from Tizard's studies, where children in high quality institutions showed only slightly lower IQ scores than their age-mates home reared children (Tizard & Joseph, 1970; Tizard & Tizard, 1971).

2.2.2. Was there any effect of age at admission at the institution in the pattern of evolution throughout institutionalization of cognitive, language, and motor functioning?

Age of the children at the entry into the orphanage as well as length of institutionalization is thought to affect the child developmental outcomes. Some studies reporting on such association pointed to more deleterious effects resulting from younger age at the admission at the institution and a longer stay (Sloutsky, 1997). Others did not find an association between children's age at the institutional admission and their intellectual development (Kaler & Freeman, 1994), while some others reported less detrimental effects for children admitted earlier, with younger children showing better results in cognitive assessments (Aboud, Samuel, Hadera, & Addus, 1991). Results from post-adoption studies revealed a linear relation between length of institutionalization and cognitive impairment of children adopted from Romanian orphanages (O'Connor et al., 2000), which was also found by Smyke and colleagues (2007), who related more cognitive compromise in older than younger children in institutions (Smyke et al., 2007). Although some were contradictory findings, we would expect that institutional care would be more detrimental for children admitted at younger ages, since institutional placement is thought to be less adequate for young children (Castle et al., 1999).

2.2.3. What was the etiological role of prenatal risk and birth weight in children's mental development?

Low birth weight, reflecting poor nutrition during fetal development, is a risk factor for neurological impairment and developmental delays (Vorria et al., 2003). According to some authors (Stein, Susser, Saenger, & Marolla, 1972 in Becket et al., 2006), the likely effects of sub-nutrition during the prenatal period on cognitive development may be attenuated and extinguished when no other risk factors are present during the prenatal life, because to some extent the fetus is protected against poor

prenatal nutrition. In our sample, the prenatal life experience seems to be more complex and multi-risky, which led us to hypothesize that poor-nutrition would be accompanied by other risk factors, and thereby both the composite measure "prenatal risk" and birth weight would be associated with cognitive development during infant's institutionalization.

2.2.4. Was mental development across time associated with child's mental status at the admission?

This question has not been thoroughly analyzed in studies with institutionalized children, however some results from studies with post-institutionalized adopted children, revealed that children who show the greatest catch-up after adoption were the ones with lower cognitive scores at the moment of adoption (Beckett et al., 2006; O'Connor et al., 2000). Thus, it is possible that similar findings may emerge in our sample, especially if the institutionalization constitutes an improvement in the rearing context, as was the case for the adopted children. Nevertheless, although some association was expected between the child's mental status at the admission and their mental outcomes throughout time spent in the institution, no specific hypotheses were formulated for this research question.

2.2.5. Was mental development associated with individual differences in the early family context, including the severity of the adversity experienced?

Some findings from the literature highlighted the deleterious effects of postnatal malnutrition on cognitive development with likely enhanced effects if it occurred in the context of severe psychosocial adversity (Wachs, 1995). Also, maltreatment experiences within the family context exert deleterious effects on cognitive development, which appear to leave its marks long after the adverse event(s) occurred (Cicchetti, 2002). Accordingly, we would expect that the adverse experiences in the family context would continue to exert effects on child's mental development. Thus, the early family-risk would be associated not only with the child's mental development at the admission at the institution, but also in later moments across time spent in the institution.

2.2.6. Was mental development associated with the quality of institutional environment and care provided in the institution?

Studies interested in understanding what kind of interventions in institutions would prevent/protect children from poor developmental outcomes, revealed that simply improving the ratios child to caregiver (Hunt, Mohandessi, Ghodessi, & Akiyama, 1976, in MacLean, 2003), or providing sensory stimulation (Broussard & Decarie, 1971, in MacLean, 2003), will result in better outcomes in terms of children's developmental competence. Indeed, Groark, Muhamedrahimov, Palmov, Nikiforova, and McCall (2005) found that by decreasing the number of children per caregiver, an improvement in children's development had occurred. Also, the stimulus deprivation theory (Casler, 1961) postulates that environments characterized by diminished physical and social stimuli may be the greatest contributor for intellectual impairments, and improvements in these dimensions of the orphanage context would result in better intellectual development. Play materials availability revealed also to be important for decreasing developmental delays in children adopted from orphanages (Morison et al., 1995). Having this in mind, we expect that the quality of the institutional care would be associated with the developmental outcomes of the child in terms of their cognitive, language and motor competence.

2.2.7. How did caregiver's sensitivity and cooperation was associated with the children's mental development?

Bowlby (1951), concerning the concept of maternal deprivation, stated that a stable attachment with a sensitive caregiver is crucial for a good socio-emotional development but also intellectual development. Based on this statement, institutionalized children cared by more sensitive caregivers would show less impaired cognitive functioning. In this respect, results from the interventional study developed by Groark and colleagues (2005) corroborate this idea in which they showed that promoting caregiver sensitivity improved the cognitive development of the children (Groark et a., 2005). Also, Smyke and colleagues (2007) found a positive association between caregiving quality and DQ, when children were assessed in interaction with

their preferred caregiver (Smyke et al., 2007). Although other studies (Vorria et al., 2003) failed to find an association between caregiver's sensitivity and infants' mental and motor functioning, we would expect to find in our study an association between these two variables.

2.3. NEUROENDOCRINE FUNCTIONING: CORTISOL PRODUCTION AND REGULATION

2.3.1. How did cortisol production and diurnal regulation evolve over the time spent in the institution, from the time of admission?

Given that the scarce literature on the effects of institutionalization reported that, while still institutionalized, children showed a flattened daily pattern of cortisol, with blunted levels in the morning and no systematic decrease over the course of the day, we would expect that the same pattern of production across the day in our institutionalized sample might occur (Carlson & Earls, 1997; Kroupina et al., 1997 in Tarullo & Gunnar, 2006). However, because the level of deprivation is not so severe in our institutions, it is also possible that the patterns of cortisol in our sample may be different comparing to the studies above. Moreover, as the withdrawn from the family and consequent admission at the institution may be viewed as a stress event, since children encounter adults and other children whom they have never met, and from whom they did not know what to expect (Cicchetti & Rogosch, 2001b; Gunnar & Donzella, 2002), we would expect that the cortisol levels at the admission moment would be the highest throughout the institutionalization period.

2.3.2. Was there any age effect in the pattern of evolution of cortisol production and diurnal regulation throughout the institutionalization period?

The HPA axis responsivity to stressors decreases around the 12 months of age (Gunnar, 2003). Thus, by the end of the first year, it becomes difficult to elevate cortisol levels in response to stressors such as brief maternal separation (Gunnar, 2003). In keeping with the literature, we expect to find differences in the child's stress response as a function of age, at least at the admission moment. Regarding the following moments

of assessment, no specific hypotheses were outlined due to the scarce information on the literature.

2.3.3. What was the etiological role of prenatal risk factors in children's cortisol levels and diurnal regulation?

Information regarding children's prenatal life is quite difficult to gather, since a great number of pregnancies in these samples developed without medical supervision. However, considering the children's case files, we could expect that these mothers were exposed to a considerable degree of stress during their pregnancies experiencing constant daily hassles and negative life events. Given that, and considering that prenatal stress is associated with preterm delivery and low birth weight, we would expect an elevated percentage of cases in our sample with low birth weight and prematurity (Wadhwa, 2005; Wadhwa et al., 2001; Wadhwa et al., 1993). Furthermore, since HPA axis may be a mediator of the effects of the prenatal exposure to stress, and prenatal stress is associated with enhanced basal levels of cortisol at different ages, we would expect that children with low birth weight would show this pattern of cortisol response (Gutteling et al., 2005; Lyons-Ruth et al., 2000; Phillips, 2007; O'Connor et al., 2005).

2.3.4. Were cortisol levels and diurnal regulation associated with individual differences in the early familial context, including the severity of the adversity experienced?

Studies have revealed that early life stress in the form of child abuse or neglect may affect individual's psychological and biological development (Cicchetti & Rogosch, 2001a). A considerable amount of research on the effects of child maltreatment indicated atypical physiological regulation of the HPA axis. For instances, for children entering a new foster family, the low levels of morning cortisol corresponded to the degree of neglect they had experienced in their families of origin (Tarullo & Gunnar, 2006). Also, studies with international adoptees revealed that children who experienced severe abuse early in life showed low levels of cortisol in the morning, and also a flatter diurnal slope, while adoptees that experienced moderate abuse had higher cortisol levels and a steeper diurnal slope (van der Vegt, van der Ende,

Kirschbaum, Verhulst, & Tiemeier, 2009). In this sense, we would expect that cortisol levels and diurnal pattern might vary according to the severity of maltreatment experienced by the child before institutionalization.

2.3.5. Were cortisol levels and diurnal regulation associated with child's neuroendocrine status at the admission in terms of diurnal cortisol production and regulation?

Despite some evidence for the dysregulation of the HPA axis in institutionalized children, no studies reported on the contribution of their status at the admission at the institution to the further outcomes. Most of the research with institutionally reared children was conducted when children were already placed with an adoptive family, or already live for long in the institution, making it impossible to measure their cortisol levels at the admission. To our knowledge, there are no studies reporting on the effects of institutionalization from the time of the child's admission at the institution. Thus, although some association was expected between the status at admission and the following moments in terms of cortisol production and regulation, no specific hypotheses were formulated for this research question.

2.3.6. Were cortisol levels and diurnal regulation associated with the quality of institutional environment and care provided in the institution?

Acknowledging that activity of the HPA axis in early human development is under strong social regulation, we would expect that being deprived of an expectable evolutionary level of care, as is normally provided by the family environment, would dysregulate the HPA axis (Gunnar & Donzella, 2002; Tarullo & Gunnar, 2006). Several studies revealed that children reared in orphanages in Russia and Romania have blunted early morning cortisol levels and no systematic decrease in levels over the course of the day (Carlson & Earls, 1997; Kroupina et al., 1997 in Tarullo & Gunnar, 2006); however there is considerable variability between institutions in the quality of the institutional environment, and thus, we would expect that cortisol levels and regulation would vary depending on the quality of the care provided in the institution. As far as we know, there is no study in the literature that investigated how the characteristics of the institutional

environment relates to cortisol production, but we could refer, from studies with children placed in day care centers, that higher levels of cortisol were found in contexts with poor adult:child ratios (Legendre and Korintus, 1996 in Dettling et al., 2000). Thus, we would expect that some characteristics of the institutional environment would be related to the child's cortisol levels produced across time spent in the institution.

2.3.7. How did caregiver's sensitivity and cooperation associate with the cortisol levels and diurnal regulation?

Several authors argue that, based on animal models, parental care impacts activity of the HPA axis, acting as a social regulator and potential buffer of the activity and regulation of the system in infants and young children (Gunnar & Donzella, 2002). In this sense, disturbances in parental care, even within the normative range of variations in parental care, may disrupt the expected functioning of the HPA stress system. Guided by the attachment theory, as in research on non-human primates, assessment of parental sensitivity/responsiveness of the caregiver toward the signals of the child would be examined as a way of testing the parental buffering hypothesis in humans (Gunnar & Quevedo, 2008). Overall, studies indicate that parental sensitivity/responsiveness buffers the stress response of the child.

2.4. RELATION BETWEEN NEUROENDOCRINE FUNCTIONING AND PHYSICAL GROWTH AND MENTAL DEVELOPMENT

2.4.1. Were cortisol levels and regulation associated with physical growth and mental development?

Assuming that psychosocial deprivation has an impact on regulation of the HPA axis and increase activity of the HPA axis, manifested by elevated levels of CRH and cortisol (which is suggested to impact on the growth axis, through inhibition of growth hormone and growth factors (Albanese et al., 1994; Cianfarani et al., 2002), we would expect to find an association between growth failure in institutionalized children and HPA axis (dys)regulation.

Also, acknowledging that cognitive deficits in post-institutionalized children have been suggested to arise from the exposure in early life to neurotoxic levels of glucocorticoids that resulted from the chronic activation of the HPA axis (Marshall & Kenney, 2009), we would expect that levels of cortisol production in our institutionalized sample is associated with delays in their cognitive development.

3. METHOD

3.1.PARTICIPANTS

Participants in this study were a subset of children and institutional caregivers drawn from a longitudinal study conducted with Portuguese children placed in temporary institutional care homes, aged between 0 to 30 months.

Children

All children were first assessed when admitted at the institution (Time 0, hereafter T0) and afterwards, in three time point assessments (Time 1, Time 2, Time 3, hereafter T1, T2, and T3, respectively), at specific developmental ages - 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 months – according to their age at admission.

The sample consists of 31 institution-reared children, 16 boys (51,6%) and 15 girls (48,4%) that remained institutionalized until T3 assessment, in 15 temporary institutional care homes, in the north of Portugal. The age of children (adjusted to prematurity) at the admission (T0) ranged from 1 to 19 months (M = 9.35, SD = 5.46). At T1, children's age ranged from 3 to 24 months (M = 11.65, SD= 5.61), and at T2 ranged from 6 to 27 months (M = 14.35, SD = 5.54). Finally, at T3 the age ranged from 9 to 30 months (M = 17.58, SD = 5.52).

Regarding the length of institutionalization at the admission in the study (T0), it ranged from 0 to 52 days (M = 9.90, SD = 11.85). At T1, length of institutionalization ranged from 1 to 4 months (M = 2.03, SD = 0.91), and at T2 ranged from 3 to 7 months (M = 4.87, SD= 1.02). Finally, at T3, assessment length of institutionalization ranged

from 6 to 10 months (M = 7.84, SD = 1.04). Descriptive statistics of the sample of children can be found in table 1.

For the sample constitution, the following selection criteria were applied: (a) age at admission ≤ 21 months (in order to be ≤ 30 months at T3 assessment); (b) age at admission above 3 months of age or, if less, having established a clear diurnal cortisol rhythm defined as a peak in the morning and a significant decrease across the day to at least half of morning levels by late afternoon (Knutsson et al., 1997; Sikes, 1992) (c) no genetic or neurological syndromes (e.g., Down syndrome); (d) no diagnosis of fetal alcohol syndrome in the medical records; (e) having completed the protocol assessment at the three time point assessments (T1, T2, T3) plus the admission assessment (T0).

During the time period of data collection - two years and a half - 73 children were admitted at the institutional care homes, but 22 of them were excluded of the present study, at different time points, for not fulfilling the selection criteria (one due to cerebral palsy and the other due to sensorial impairment; three because the admission age was above 21 months; nine because the admission age was below 3 months and the diurnal cortisol rhythm was not yet established; six because of incomplete cortisol assessment at T0, one at T1, and another at T2), 11 were reintegrated in their biological family before T3 assessment, eight were adopted before T3 assessment, and one left the institution to be reintegrated in another institution before T3 assessment. Figure 1 depicts the flowchart of dropouts during the period of data collection and according to the exclusion criteria.

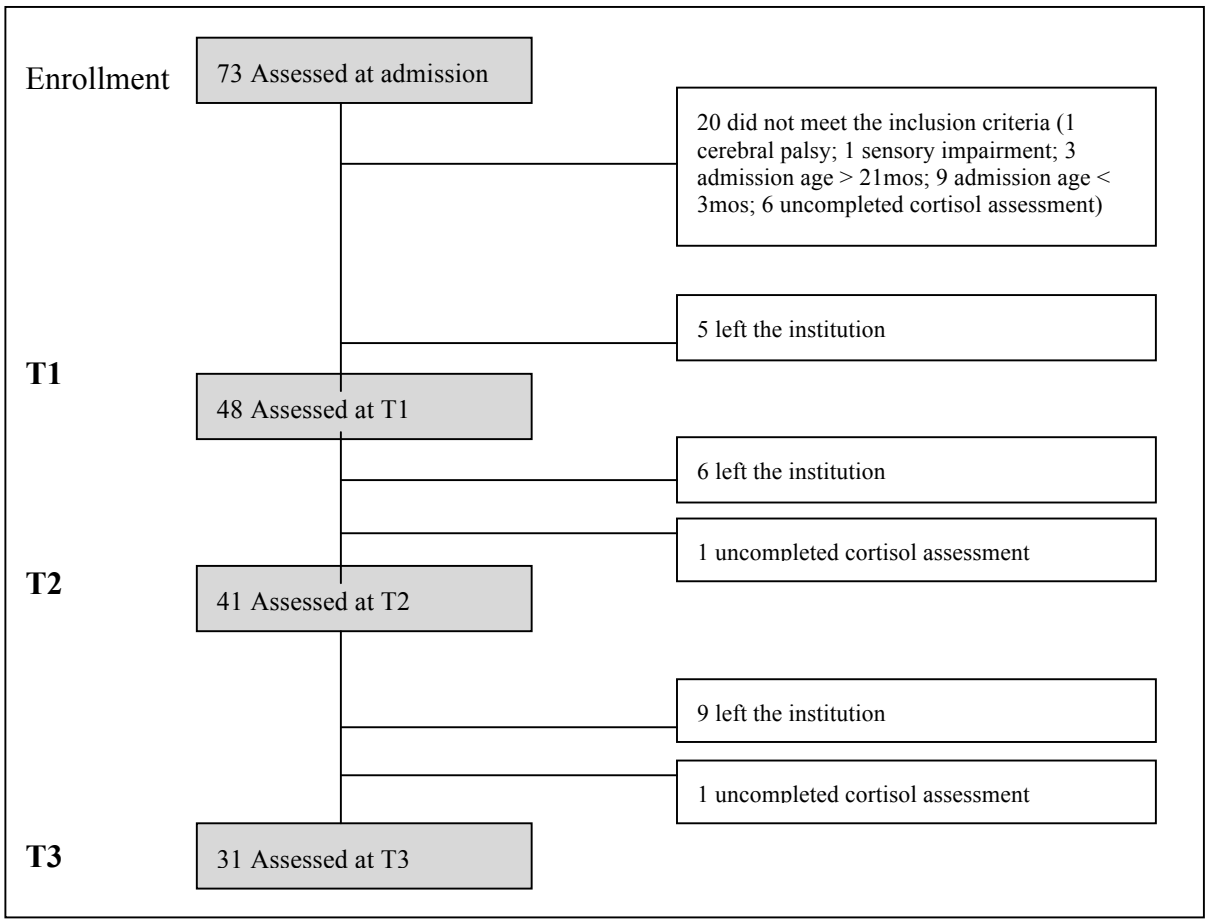

Figure 2: Flowchart depicting the dropouts during the period of data collection and according to exclusion criteria

Regarding the reasons for the children's admission at the institutional setting, an aggregation into eight categories were proposed as follows (due to the wide range of events that precipitated the withdrawn from the family): child's abandonment, negligence, lack of parental skills, lack of socioeconomic conditions, parental psychopathology or mental retardation, child's physical abuse, child's sexual abuse, and family or domestic violence. Thus, the most common motives presented for children's admission at the institution were lack of parental skills, which may refer to teenage parenting, parents already known and referenced by social services as incapable of properly caring for their children in the past, or parents known to be substance abusers and negligence. In fact, nine children (29.03%) were admitted due to this reason. Also nine children (29.03%) were admitted at the institution because of failure to provide minimum standards of care as well as parents inability to ensure safety needs. In turn, seven children (22.58%) were admitted because of abandonment, of which one was

abandoned at birth. Two children (6.45%) were admitted due to exposure to family or domestic violence, and other two (6.45%) due to sexual abuse. One child (3.23%) was admitted due to parental psychopathology or mental retardation, and other one (3.23%) due to physical abuse. None of the children included in this subsample were admitted due to lack of socioeconomic conditions. Data presented above reflects the distribution of the children in terms of the main reason for their admission at the institution, which doesn't mean that they were not exposed to other previous risk conditions. In fact, 23 children (74,19%) were exposed to more than one of the eight categories of risk described above. According to the nosological classification system for child maltreatment proposed by Barnett and colleagues (1993), 17 children (54.84%) were maltreated before admission at the institution, while the remaining 14 (45.16%) were not.

Furthermore, it is important to note that prenatal and neonatal risk factors were not considered above, despite their relevance in the life histories of these children. In fact, according to the information available, 11 biological mothers used drugs of abuse during pregnancy (35.50%; data only available for 29 participants), seven exposed their children to alcoholic substances (22.60%; data only available for 29 participants), and eight used tobacco while pregnant (25.80%; data only available for 29 participants). There are six (19.40%) preterm infants in this subsample, and eight children had low birth weight (25.80%; LBW < 2500 g; data available for 30 participants). One child was admitted at the institution directly from the maternity hospital (3.20%, data available for 30 participants).

Table 1

Children's social, health and demographic characteristics

Age and length of institutionalization	***M (SD)***	**Range**
Age at T0	9.35 (5.46)	1 - 19
Age at T1	11.65 (5.61)	3 - 24
Age at T2	14.35 (5.54)	6 - 27
Age at T3	17.58 (5.52)	9 - 30
Length of institutionalization at T0 (days)	9.90 (11.85)	0 - 52
Length of institutionalization at T1 (months)	2.03 (0.91)	1 - 4
Length of institutionalization at T2 (months)	4.87 (1.02)	3 - 7
Length of institutionalization at T3 (months)	7.84 (1.04)	6 - 10
Reason for admission at the institution	**N**	**%**
Lack of parental skills	9	29.03
Negligence	9	29.03
Child's abandonment	7	22.58
Parental Psychopathology/mental retardation	1	3.23
Family violence	2	6.45
Sexual abuse	2	6.45
Child's physical abuse	1	3.23
Preterm delivery	**N**	**%**
Yes	6	19.40
No	25	.80.60
Low Birth Weight (LBW)	**N**	**%**
Yes	8	25.80
No	22	71.00
Missing information	1	3.20

Institutional Caregivers

Institutional caregivers were requested to participate in the study according to the following guidelines: (a) at the admission (T0) none of the institutional caregivers knew the child, so the institutional technical staff was inquired about the existence of a primary caregiver assigned by them for that specific child, who would be invited to participate in the study; (b) if there was no assigned caregiver, the participation from one of the caregivers that usually takes care of the group of children to where the admitted child was assigned, was requested; (c) at T1, T2 and T3 assessments, both the technical and the caregivers team were questioned about the existence of a specific

caregiver who spent more time or more frequently looked after the child, or who felt more committed to that child; if it was possible to identify a caregiver, he/she was asked to participate in the study; if it wasn't possible or if the former caregiver was still the primary caregiver, the former caregiver was requested to continue participating in the study. Accordingly, some changes occurred from moment to moment in the caregivers participating in the study.

At the admission time (T0), 24 different institutional caregivers participated in the study, in which 5 caregivers participated paired with two different children and 1 caregiver participated paired with three different children. Most of the caregivers were female (95.65%), and their mean age was 36.38 years (*SD*= 12.00). At T1, 26 different caregivers participated in the assessments, five of which participated paired with two different children. Their mean age was 34.41 years (*SD*= 10.51) and most of them were female (92.00%). At T2, 26 different caregivers were enrolled in the study, five of which participated paired with two different children. The mean age of the caregivers at this time point was 34.19 years (*SD*= 10.69) and 92.31% of them were female. Finally, at T3, 25 different caregivers participated in the assessments, in which six participated paired with two different children. Most of the caregivers were female (92.00%) and their mean age was 34.00 years (*SD*= 10.40). The minimum age of the caregivers was 20 years and the maximum was 60 years.

Regarding formal educational, most of the caregivers completed 9th grade (compulsory education) or were graduated from high school. At each assessment point, we observed that the majority of caregivers did not receive specific training to perform their work in the institutions, exception made at T0 where the number of caregivers who received and not received specific training was equal. At T0, caregivers participating in the study worked as an institutional caregiver for a mean time of 4.82 years (*SD*=4.78). At T1, the mean was 5.01 years (*SD*=4.67), at T2 was 5.12 years (*SD*=4.46), and T3 the mean time was 5.32 years (*SD*=4.50). The minimum time of work as an institutional caregiver was 3 months and the maximum time was 18 years.

It should be noted that data included here refer only to the caregivers who participated in the interaction procedures with the child, since this is the only moment where they were also evaluated in terms of their sensitivity and cooperation with the

child. Missing data does exist because some interaction videotapes were not possible to code.

Table 2
Caregiver's socio-demographic and professional qualification information

		T0		T1		T2		T3	
		(N=24)	%	(N=26)	%	(N=26)	%	(N=25)	%
Gender	Female	23	96.2	24	94.4	24	94.7	23	94.6
	Male	1	3.8	2	5.6	2	5.3	2	5.4
Age	20-35 years	11	45.83	15	57.69	17	65.38	15	60.00
	36-60 years	9	37.50	10	38.46	9	34.62	9	36.00
	Missing information*	4	16.67	1	3.85	-	-	1	4.00
Formal Education	Primary school	-	-	2	6.45	4	12.90	1	3.23
	6th grade	1	3.23	2	6.45	2	6.45	3	9.68
	9th grade	14	45.16	15	48.39	13	41.93	11	35.38
	High school grad.	9	29.03	8	25.82	10	32.26	10	32.26
	University grad.	2	6.45	1	3.23	1	3.23	2	6.45
	Missing information*	5	16.13	3	9.68	1	3.23	4	12.90
Specific training	Yes	15	35.50	15	48.39	13	41.90	13	41.94
	No	11	48.40	14	45.16	18	58.10	16	51.61
	Missing information*	5	16.10	2	6.45	-	-	2	6.45
Years of work	0-5 years	22	71.00	21	67.70	21	67.70	22	71.00
	6-9 years	3	9.70	4	12.90	7	22.60	5	16.10
	10-20 years	1	3.20	4	12.90	3	9.70	2	6.45
	Missing information*	5	16.10	2	6.50	-	-	2	6.45

* Due to the fact that the caregivers didn't completely filled or return the questionnaire.

3.2. MEASURES

Three main foci of assessment were designed *a priori* for the study: the child, the interaction between child and institutional caregivers, and both the child's history and early family context and the institutional rearing context. According to these foci some measures were selected, which are described below, to assess specific domains of

child's development, caregiver's competence when interacting with the child and quality of early family and institutional contexts.

Child assessment

Physical Growth

Data on physical growth – height, weight and head circumference – were obtained from children's medical records, at each moment of assessment (T0, T1, T2, T3). Information on birth anthropometric measures was also available from medical records, for most of the children (measure of birth weight and height were missing for one child, and head circumference for two children). To allow the comparison between children of different ages, all anthropometric measures, height (supine length < 24 months; or standing height ≥ 24 months), weight and head circumference, were converted to percentiles and age-standardized scores (z scores) using the software program WHO Anthro Statistical Software version 3.1 (2010; http://www.who.int/childgrowth/en/). Anthropometric measures are reported in standard deviation units. Growth delay for height, weight, and head circumference was defined as standardized scores ≤ -2. In cases of known prematurity, physical measurements at birth were excluded from analyses.

Mental Development

For the child's mental development assessment, the Bayley Scales of Infant and Toddler Development (3rd edition; Bayley, 2006) were used. This is an individual measure to assess the developmental functioning of infants and toddlers, aged between 1 and 42 months. The battery is arranged into three administered scales - the Cognitive Scale, the Language Scale (divided into Receptive Communication and Expressive Communication subtests), and the Motor Scale (divided into Fine Motor and Gross Motor subtests), that were carried out by trained examiners. Depending on the age of the child, the administration can last from 30 minutes up to 1 hour. However, it is not mandatory to administer the battery of tests all at a time. Instead, it should be distributed into smaller periods of time, according to the child's alertness state, ability to focus attention, and tiredness, to allow getting the child's best performance in all items. To

determine the child's start point, his/her chronological age was computed, adjusting for prematurity up until 24 months, and then the starting point was selected matching his/her age.

Each scale is composed by several items of growing difficulty. The cognitive scale is composed by a total of 91 items, and allows for the assessment, among other relevant domains, of children's sensorimotor development, exploration and manipulation, concept formation, and memory. The subscale of receptive communication, from language scale, includes 49 items designed to assess preverbal behavior, morphological and vocabulary development, while the subscale of expressive communication includes 48 items conceived to assess preverbal communication such as babbling, gesturing, naming objects and pictures, and also morpho-syntactic development such as compose sentences. Fine motor subscale consists of 66 items designed to assess skills related to perceptual-motor integration, while the gross motor subscale is composed by 72 items intended to assess, among others skills, locomotion, coordination and balance skills.

A raw score is obtained for each of the three subscales, cognitive, language and motor development, which are then converted into percentiles. For the expressive and receptive language subscales, and fine and gross motor subscales, a composite score is obtained, though not a percentile. The original validation study included a sample of 1,700 children aged from 16 days to 43 months and 15 days, divided into 17 age groups with 100 participants each. The inter-scorer reliabilities were very high, ranging from 0.98 to 1.00 for the five subtests across all age groups, as well as the internal consistency reliabilities, which are 0.91 for Cognitive Scale, 0.93 for Language Composite Scale and 0.92 for Motor Composite Scale.

Neuroendocrine functioning – Daily Cortisol Production and Regulation

Saliva samples were collected to determine salivary cortisol levels. Salivary cortisol proved to be a valid and reliable reflection of the respective unbound hormone in blood for children and adolescents (Woodside, Winter, & Fisman, 1991). Samples were collected at T0, T1, T2 and T3, in the morning, noon and afternoon during one single day. Specifically, we collected saliva in three blocks of times. Block one implies collecting saliva between 7 and 9 a.m., when the child was awake for at least 30 minutes

(sometimes it was not possible due to institutional routines restrictions), non-distressed and before being fed (hereafter Wakeup). Block two occurred between 11 a.m. and 1 p.m., when the child was awake for at least 30 minutes, non-distressed and before being fed at lunch (hereafter Noon). Block three implies that saliva was collected between 5 and 7 p.m., when the child was awake for at least 30 minutes, non-distressed and before being fed or at least 30 minutes after being fed (hereafter Afternoon). Saliva was collected using a cotton roll "Salivette" (Sarstedt, Nümbrecht, Germany) that we introduced into the mouth of the infant and asked him/her to chew it or, when it was not possible due to their early ages, we repeatedly pass the cotton roll in the cheeks, where the production of saliva is more intensive, for about 2 minutes or once the cotton roll was saturated, and then returned it into the vial. Each vial was labeled with the date and time of collection and child's code. Samples were maintained cool, at 4ºC, until handled in the laboratory. They were centrifuged, within up to five days, for 12 min at 3200 rpm and then transferred into a 1.5 ml Eppendorf Safe-Lock microtubes to be next stored at -80°C, until assayed. These procedures have been shown to have no effect on cortisol values (Clements & Parker, 1998).

We avoided collecting saliva when infants were ill, and also when, for some reason, they were excited or distressed. For instances due to vaccination, visits from the biological parents, or procedures that included contact with strangers.

Samples were assayed using the IBL Kit for Cortisol Saliva ELISA (IBL, Hamburg, Germany). Samples were assayed in duplicate with all samples from a subject placed in the same assay batch to prevent inter-assay variation that could interfere with the results. Assay batches were constructed so that group differences would not be a reflection of inter-assay variation. The values were scanned for ones that appeared physiologically improbable (i.e., >4.0 µg/dl). Inter- and intraassay coefficients of variation were below 7 and 4%, respectively.

Due to institutional routines constraints, the collection of saliva at 7am was not performed for all children 30 minutes after they had woken up, thus a preliminary analysis was conducted to assess whether there was an association between the cortisol levels at 7am, and the time since the child was awake. Results revealed no association between the two variables at T0, $r_s = -.002$, $p = .99$, T1, $r_s = -.12$, $p = .48$ T2, $r_s = -.05$, $p = .78$, and T3, $r_s = -.13$, $p = .42$.

The area under the curve with respect to ground (AUCg) was calculated for all children in all moments of assessment. This is a useful and frequently used method in endocrinological research and the neurosciences as it allows comprising information contained in repeated measurements over time (Pruessner, Kirschbaum, Meinlschmid, and Hellhammer, 2003). The formula for calculating the AUCg can be derived from the trapezoid formula and it calculates the total area under the curve of all the measurements as the area of interest (Pruessner et al., 2003). This formula considers the difference between the single measurements from each other (i.e., the change over time) and the distance of these measures from the ground, or zero (i.e., the level at which the changes occur over time).

Cortisol regulation was measured as the difference between the morning levels and afternoon levels. It represents the magnitude of the decrease (or increase) in the cortisol levels across the day.

Child's Life History and Early Family Context

Familial risk composites

A socio-demographic questionnaire was designed to this study to capture some aspects regarding infant's life before institutionalization, and also information about their biological families. It includes issues such as reason for admission in the institution, housing conditions and household composition (where and with whom the child lived before institutionalization), socioeconomic status (SES) of the biological family, health history of biological parents, pregnancy and delivery history, and health and developmental history of child before admission at the institution. Both the child's individual health records and social file were consulted in order to fill out the questionnaire. Also, the institutional technical staff who knew the child well were inquired for additional information that was absent in the files. Individual social files revealed to have a considerable amount of missing data in some cases, which for many of them were also not available from the institutional staff.

In addition, three theoretically oriented composites were created for each child, on the basis of the collected data, representing early family risk factors. When more than 25% of the variables that forms the composite were missing for a subject, it was established that a composite score was not assigned for that case. Conversely, when

75% of the variables were present, the cumulative score was divided by the total existing variables, which ends up in a score ranging from 0 to 1 that was given to each subject included in each risk composite, indexing the proportion of risk components present for a given composite.

Prenatal risk composite: it is composed by four variables, coded 0 or 1, according to the absence or presence, respectively, of the following risk factors: maternal physical disease (e.g. AIDS, Hepatitis); maternal substance abuse during pregnancy; pregnancy without medical surveillance; and prematurity.

Family-relational risk composite: the following risk factors, coded 0 or 1 according to their absence or presence respectively, contributes to this composite: government aid recipient; domestic violence (to the children and/or between parents or other family members living in the house); family previous referral by the social workers as a risk family (based in conditions such as maltreatment, negligence or abandonment of other children); and institutionalized or adopted siblings.

Emotional-negligence risk composite: it comprises the following four risk factors, coded 0 or 1, according to their absence or presence respectively: negligence as the reason for admission to the institution; maternal prostitution; maternal substance abuse; and maternal psychopathology or mental retardation. This composite was created in an attempt to capture the likely unavailability of the maternal figure.

Child-Caregiver interaction assessment

Caregiver's sensitivity

The sensitivity of the caregiver's behavior in interaction with the child was observed in an interactive procedure, consisting of three episodes, with 3/5 minutes each, where three different tasks were given: (1) The caregiver was asked to play freely with the child, while making use of the toys/materials available from the Bayley III scales Bayley, 2006); (2) The caregiver was asked to play with the child without toys,

that were removed by the researcher in the meantime; (3) The caregiver and child were asked to play with a toy that is too difficult considering the age of the child (different toys were used in each age group) and caregivers were instructed to help the child in the way they usually did. All interactions were videotaped and subsequently scored by trained coders. The caregivers' sensitivity and cooperation were measured using the 9-point Sensitivity/Insensitivity and Cooperation/Intrusiveness subscales from the Maternal Sensitivity Scales (Ainsworth et al., 1978).

The Sensitivity/Insensitivity subscale assesses the caregiver's ability to interpret accurately the signals and communications implicit in infant's behavior, and given this interpretation, to respond to them appropriately and promptly (Ainsworth et al., 1978). The highest score in the scale, 9, corresponds to a highly sensitive caregiver, while a score of 5 corresponds to an inconsistently sensitive caregiver and the lowest score, 1, corresponds to a highly insensitive caregiver. In the present study, an overall sensitivity score was attributed to the whole interactive procedure (12/15 minutes).

The Cooperation/Intrusiveness subscale deals with the extent to which the caregiver's interventions are initiations of interaction that break into or interrupt the infant's ongoing activity (Ainsworth et al., 1978). The highest score in the scale, 9, corresponds to a conspicuously cooperative caregiver, while a score of 5 corresponds to a mildly interfering caregiver and the lowest score, 1, corresponds to a highly interfering caregiver. This subscale was rated separately for the three episodes of the interactive procedure.

At T0, the mean score for sensitivity was 3.36 (*SD* = 1.44) and for cooperation was 3.88 (*SD* = 1.49). At T1 the mean scores for caregiver's sensitivity and cooperation were 3.50 (*SD* = 1.71) and 4.04 (*SD* = 1.55), respectively. Sensitivity mean score at T2 was 3.65 (*SD* = 1.58) and cooperation score was 4.06 (*SD* = 1.29), while at T3 were 3.70 (*SD* = 1.70) and 4.06 (*SD* = 1.44), respectively.

The average intra-class correlation (ICC) for inter-coder reliability (established for around 30% of the sample) was for sensitivity, ICC = .91, and for cooperation, ICC = .90).

Institutional context assessment

Quality of institutional context

The quality of institutional care was assessed using the Assessment of the Quality

of Institutional Care (AQIC, Silva, Baptista, Marques, Oliveira, Oliveira, & Soares, 2010). Structural and relational aspects of the quality of institutional care were the focus of researchers' systematic observations throughout two years of data collection at the institutions. Two main dimensions were highlighted and assessed: (a) institutional resources and routines in terms of human resources (6 items), equipment and material resources (13 items) and basic needs routines (4 items); (b) institutional relational care including the developmental activities promoted by the technical and caregivers staff (4 items), stability and consistency of caregiving (5 items), and responsiveness to the child's stress signals (1 item). Each dimension was rated on a 5-point scale (1 – no/never present; 3 – sometimes/somewhat present; 5 – yes/always present). A total score for each dimension, sub-dimension and overall quality of the institutional context was obtained, by summing the corresponding items.

Inter-rater agreement was calculated based on intra-class correlations and proved more than adequate for all two dimensions of AQIC: institutional resources and routines (ICC mean r_{ic}= .84, range= .64 - .97), institutional relational care (ICC mean r_{ic}= .83, range= .75 - .88). Inter-rater reliability was calculated for 31.60% of the institutional settings.

Table 3: Measures, Aims and Time of assessment

	Measure	Aim of assessment	Time
Child	Medical Records (Direcção Geral de Saúde, 2005)	Physical growth: weight, height and head circumference	T0, T1, T2, T3
	BSID III – 3rd ed. (Bayley, 2006)	Mental development: cognitive, language and motor development	T0, T1, T2, T3
	Salivary cortisol levels	Diurnal production of cortisol	T0, T1, T2, T3
Life history & Early family context	Socio-demographic questionnaire	Familial risk composites: Prenatal risk composite; Family-relational risk composite; and Emotional-negligence risk composite	T0
Child-caregiver interaction	Maternal Sensitivity Scales (Ainsworth, et al., 1978)	Caregiver's sensitivity and cooperation	T0, T1, T2, T3
Institutional context assessment	AQIC (Silva, Baptista, Marques, Oliveira, Oliveira, & Soares, 2010)	Quality of institutional context and care	T0 – T3

4. PROCEDURE

The study here presented is part of a broader study with Portuguese institutionalized and adopted children, aged between 0 to 30 months. The study was approved by the Portuguese Institute of Social Security, which was contacted by the research team in order to get its permission to conduct the research in their Temporary Institutional Care Homes. The National Commission for Data Protection also gave its permission for the study to be conducted. The study and all the procedures were presented to the director and technical staff of each institution, in a meeting scheduled to ask their permission and cooperation in the research. An informed consent was signed by each institutional director in which he/she states his/her permission to the performance of the research in his/her institution. The technical staff of each institution was committed to inform our research team whenever a new child, aged between 0 to 24 months, was admitted to the institution. This assessment at the admission time allowed

us to get a baseline assessment that marks the transition between two different contexts, and somehow isolates the institutionalization effects. This was particularly interesting to the cortisol measurement as it was expected that the withdrawn from the family and the entry into a new context, where children encountered adults and children whom they had never met before, and from whom they did not know what to expect, constituted a stress-eliciting event (Cicchetti & Rogosch, 2001b; Gunnar & Donzella, 2002).

At the admission moment, we started by consulting children's individual files and medical records in order to ascertain his/her eligibility to the study. All the children admitted in the institution were eligible with exception made for the children aged more than 24 months at admission, with severe sensorial impairments, genetic or neurological syndromes (e.g., Down syndrome) and diagnosed with fetal alcohol syndrome in the medical records. For all the children, an informed consent was obtained from their biological parents when they still maintained contact with the child (by visiting him/her at the institution) after they were elucidated about the main goals and procedures of the study and agreed with them. When the contact with the biological parents was broken (e.g., due to legal determination) or non-existent because parents abandoned the children or are unaccounted for and the institution could not reach them, it was the director of the institution who signed the consent for the child's to participate in the study.

After deciding the children's enrollment in the study, the technical staff was asked about the existence of a primary caregiver assigned by them to this particular child. If the institutional staff doesn't assign a caregiver to each child at the entry in the institution, the research team inquired the staff if there was any caregiver who has spent more time with the child during his/her first days at the institution. It is important to note that the participation of the caregivers in the assessment at the admission moment, was only required several days after the children's admission, thus this allowed that technical staff was able to determine whether there was a caregiver who spent more time with the child during these first days. If there was not a caregiver who spent more time with the child, a caregiver who usually takes care of that child and was present in children's daily routines was selected to integrate the study. Some caregivers were assigned to more than one child. Caregivers were given an explanation of the aims and procedures of the study, and then invited to participate. An informed consent was signed by them when they agreed to participate. In the following moments of assessment (T1,

T2, T3) technical staff were repeatedly asked whether there was a particular caregiver who spent more time with the child or more frequently looked after the child, who knew the child better and/or who felt more committed to the child. If the staff was able to identify such a caregiver, this suggestion was individually checked by a member of the research team through naturalistic observations of daily routines. When the staff and research team could not identify a caregiver who met the above criteria, the caregiver that participated in T0 assessments was invited to continue participating in the study. As a consequence some changes in the caregivers participating in the study occurred from moment to moment.

All the procedures, corresponding to each time of assessment (T0, T1, T2, and T3), were conducted within the institutional setting. At the admission, child's assessment was carried out as close as possible to the admission time, in an available room designated by the institutional staff, and known to the child (usually in his/her room or in an activity room). When needed, the caregiver assigned to the child was encouraged to participate in the assessment procedures in order to get the best response from the child. After the child's individual assessment has ended, we proceeded with the caregiver-child interaction assessment, which was scheduled according to the caregiver and institution availability, in order not to disrupt the institutional routines and overwhelm the caregivers, the child and the institution. In the following moments, all the assessments were conducted in accordance with the guidelines outlined above.

CHAPTER 3

PHYSICAL GROWTH, MENTAL DEVELOPMENT AND NEURO-ENDOCRINE FUNCTIONING IN PORTUGUESE INSTITUTIONALIZED CHILDREN: A LONGITUDINAL STUDY.

RESULTS

All the analyses were conducted using IBM SPSS, version 19.0. Significance for all analyses was set at $p < .05$, although marginally significance was also considered and was set up at $p < .10$. Nonparametric tests were used since for most of the variables (exception for height and head circumference) the assumptions to run parametric tests were not fulfilled. In order to be coherent, we decided to run all the analyses based on nonparametric statistics. All the analyses were run with the total sample (N=31); when this was not the case, a reference to the sample size was made. It should be noted that this is an innovative study and exploratory in nature, with several limitations that made not possible to run complex analyses, such has regression analysis and partial correlations.

Preliminary analyses were run for the purpose of defining the composition of the sample since the daily rhythm of cortisol production was estimated to be only established around the age of three months (Price et al., 1983). Assuming that a social regulation of the cortisol levels does exist, we would expect that establishing the rhythm within the context of institutionalization would be different from having done that outside this context. Thus, infants entering the institution before or after the age of three months were expected to have different levels and patterns of cortisol production and this was taken in consideration when defining the sample.

In order to analyze the effect of age at admission at the institution (below or above 3 months) on the subsequent pattern of evolution of cortisol production across time spent in the institution, we divided the sample in two age groups. The division that follows was based on the assumption that the typical daily rhythm of cortisol production is established around 3 months of age (Price et al., 1983), and, thereby, we determined that the absence of an established diurnal pattern together with being less than 3 months old at the admission would constitute one group, and children with more than 3 months would constitute the other group. Table 4 presents mean values of AUCg (Area Under the Curve with respect to ground) for the two age groups in each moment of assessment.

Table 4

Mean values of AUCg for the two age groups

	AUCg T0 *Mean (SD)*	***AUCg T1*** *Mean (SD)*	***AUCg T2*** *Mean (SD)*	***AUCg T3*** *Mean (SD)*
Age at admission below 3 months (n = 9)	4.71 (3.02)	3.10 (1.75)	1.64 (1.04)	2.12 (.90)
Age at admission above 3 months (n =31)	4.77 (3.71)	2.43 (1.30)	2.74 (1.89)	3.04 (1.55)

For the group whose age at admission was below 3 months, the value of AUCg at T0 was 4.71 (*SD*= 3.03), at T1 was 3.10 (*SD*= 1.75), at T2 was 1.64 (*SD*= 1.04) and at T3 was 2.12 (*SD*= .90). For the group admitted above 3 months of age, the value of AUCg at T0 was 4.77 (*SD*= 3.71), at T1 was 2.43 (*SD*= 1.30), at T2 was 2.74 (*SD*= 1.89), and at T3 was (*SD*= 3.04).

Regarding differences across time in terms of cortisol production, for the group admitted below 3 months these differences were only marginally significant, χ^2 (3) = 6.47, $p < .10$. While, for the group admitted above 3 months of age the differences across time spent in institution were significant, χ^2 (3) = 20.16, $p < .001$. Figure 3 depicts the evolution for the two age groups.

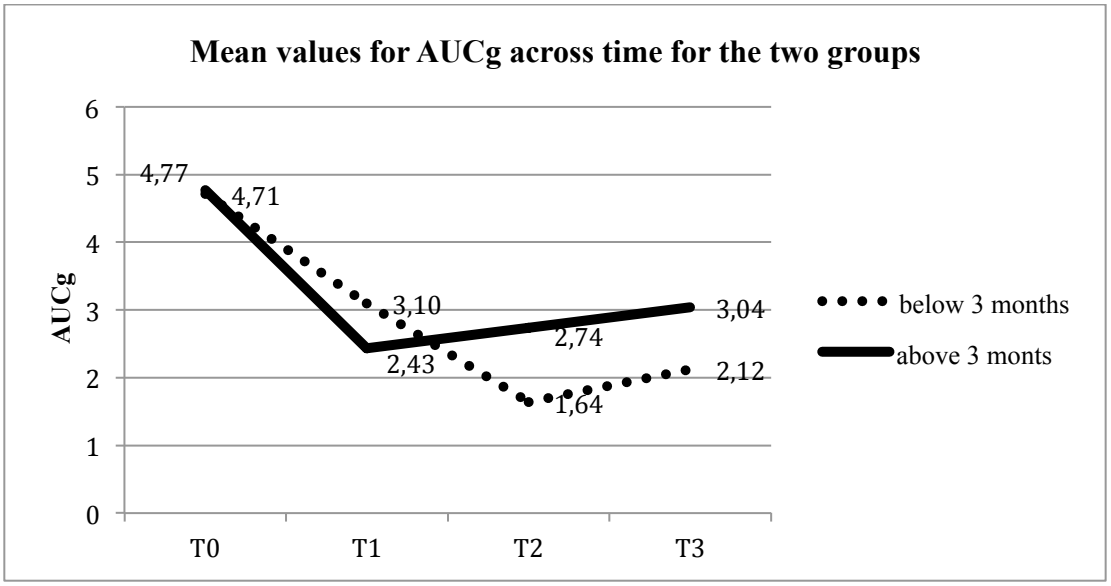

Figure 3: Evolution across time for the two age groups

The table that follows (Table 5) presents where the differences between moments of assessment occurred for each age group. For the group below 3 months of age at admission, there was a significant decline in cortisol production between T0 and T2, and T0 and T3, while for the group admitted above 3 months of age there was a significant decline between T0 and T1, $Z = -3.37$, $p < .01$. Differences between T0 and T2 remain significant, $Z = -3.31$, $p < .01$, whereas between T0 and T3, due to the increases from T1 to T3, differences are only marginally significant, $Z = -1.94$, $p < .10$. This result suggests that in about 8 months of institutionalization there was an increase in cortisol production for the group admitted above 3 months of age, approaching baseline levels. The significant increase occurs between T1 and T3, $Z = -2.16$, $p < .05$.

Table 5

Differences between moments of assessment for each age group

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
Below 3 months	*ns*	*	*	*ns*	*ns*	*ns*
Above 3 months	**	**	†	*ns*	*	*ns*

** $p < .01$; * $p < .05$; † $p < .10$

Concerning the differences between the two groups, they emerged at T2 and T3. At T2, children admitted below 3 months of age produced less cortisol during the day than children admitted above 3 months of age, $U = 79.00$, $p = .051$. At T3, there were also differences between groups, wherein children admitted below 3 months produced less cortisol than children admitted above 3 months of age, $U = 86.50$, $p = .086$. In other words, children who have established their diurnal cortisol rhythm within the context of the institution were producing less cortisol after around 5 and 8 months of institutionalization, than children who were admitted at later ages. Because of this age effect, we decided to remove from the sample all the children from the group “age at

admission below 3 months" in order to avoid having children that established their cortisol rhythm at the institution. Thereby, all the following analyses were run with a size sample of 31 children, exception made for some variables signaled by the author.

1. PHYSICAL GROWTH

The first research question that we aimed to answer in our study concerns the evolution across time of physical growth in terms of height, weight and head circumference growth.

1.1. Evolution across time for height, weight and head circumference

Height

In figure 4 the height z scores for each moment of assessment are presented. In every moment of assessment the child's growth in terms of height was more than one standard deviation below the mean expected for their age. At T0 the mean was -1.01 (*SD* = 1.85), at T1 it was -1.02 (*SD* = 1.61), at T2 it was -1.18 (*SD* = 1.56), and finally at T3 it was -1.01 (*SD* = 1.69). Additionally, at T0, 29.00% of the total sample (9 children) were more than two standard deviations below the mean expected for their age, which represents a growth delay, while at T1, T2 and T3, this percentage decreases to 22.58% (7 children).

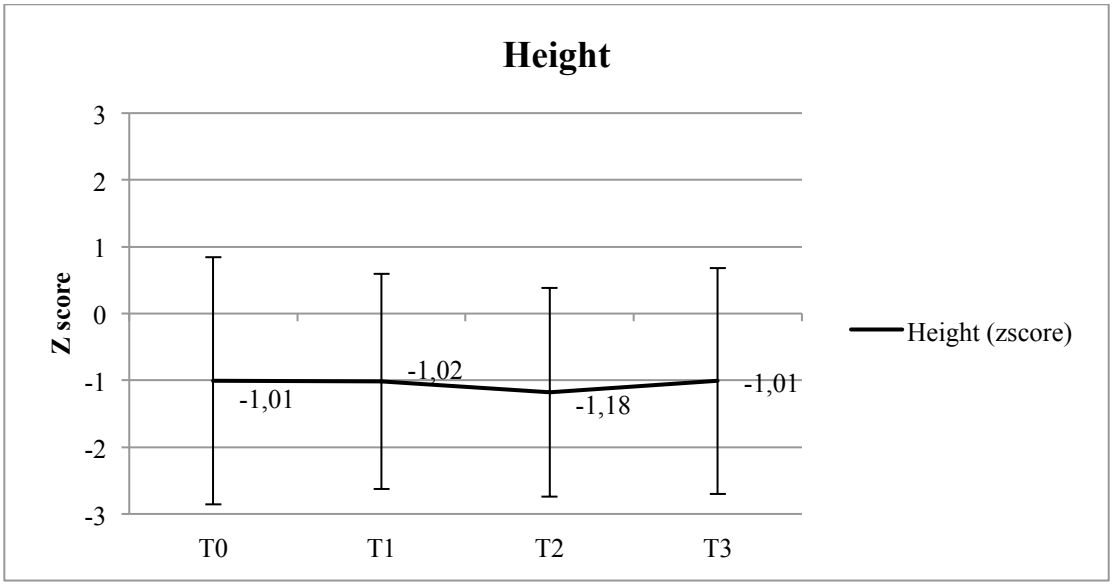

Figure 4: Evolution of child's height z scores across time

Besides that, there is no gain in terms of height growth, across time spent in institution, χ^2 (3) = 4.26, p = .24.

In order to analyze intra-individual changes the differences between T0 and T3[1] for every child were calculated and then plotted as follows. Figure 5 presents the wide variability in the magnitude of losses and gains across time spent in the institution. 14 children (45,16%) presented losses in terms of height growth between T0 and T3, while 17 children (54,84%) presented gains in height growth across time spent in the institution. In other words, the status at the admission at the institution, in terms of height, was better, for 14 children, than approximately 8 months later, while for 17 children their physical status in terms of height, 8 months after the admission, was better than at the admission.

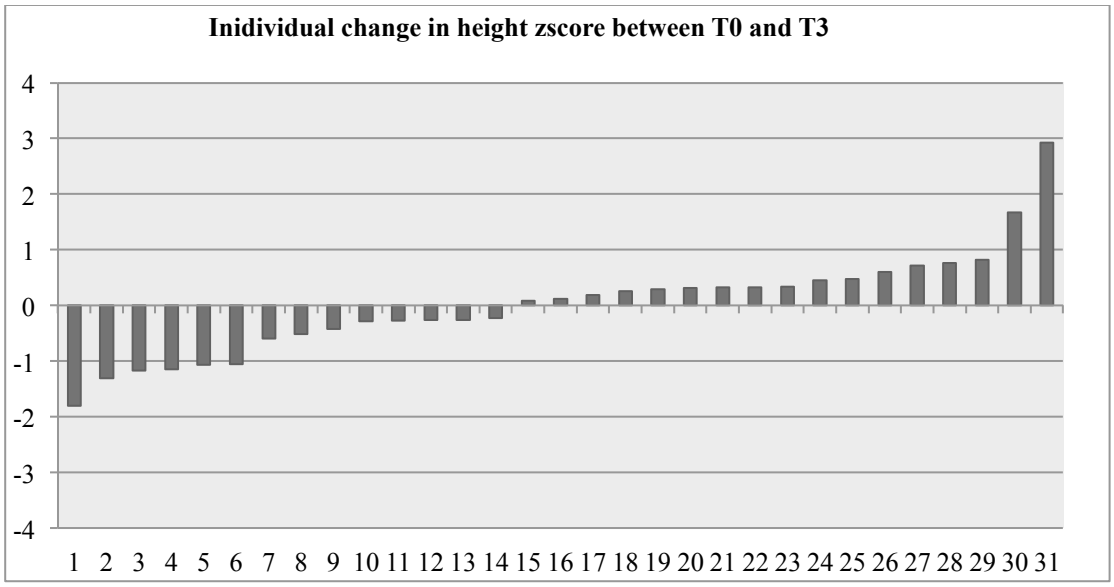

Figure 5: Intra-individual changes in height z scores across time

Weight

In figure 6 the weight z scores are presented for each moment of assessment. At T0 the mean weight z score was -0.98 (*SD* = 1.73), at T1 the mean was -0.61 (*SD* = 1.72), at T2 it was -0.46 (*SD* = 1.70), and finally at T3 it was -0.26 (*SD* = 1.68). At T0 six of the children (19.35%) showed growth delay in weight, being more than two

[1] Difference between T0 and T3 was calculated as follows: Height z score at T3 – Height z score at T0. Positive differences mean that at T3 the height z score values were higher than at T0. Negative differences mean the opposite.

standard deviations below the mean expected for their age, while at T1 five children (16.13%) were more than two standard deviations below the mean, and at T2 and T3 this percentage decreases slightly to 12.90%, with four children being more than two standard deviations below the mean.

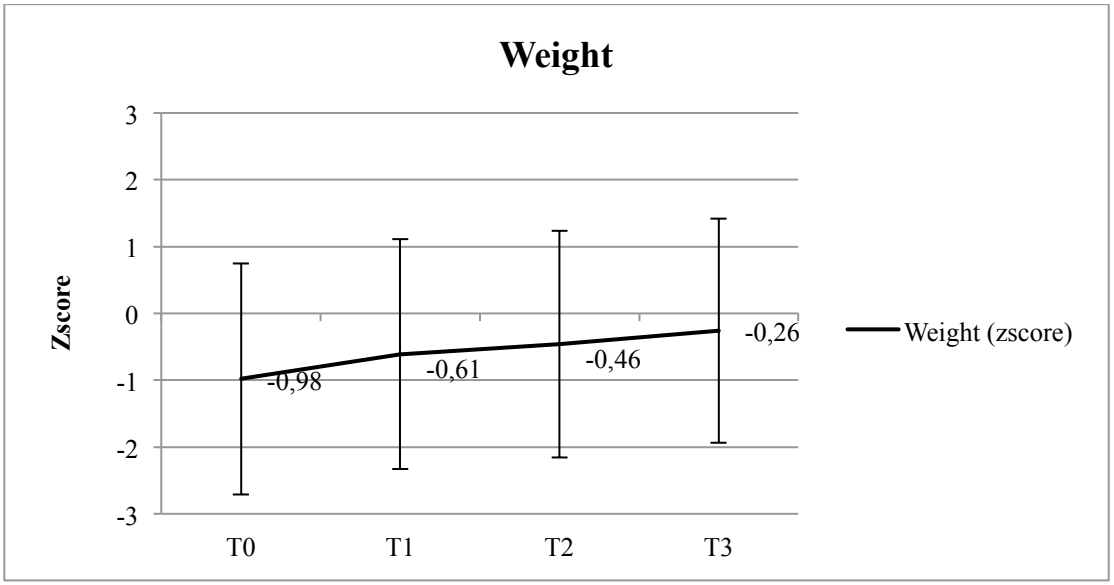

Figure 6: Evolution of child's weight z scores across time

When the main effect of time was analyzed, results revealed that there was significant gain in terms of weight growth, across time spent in institution, χ^2 (3) = 16.33, p = .001. Table 6 presents the comparisons between moments of assessment for weight z scores.

Table 6

Comparisons between moments of assessment for weight

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
Weight (zscore)	**	**	***	*ns*	*	†

*** $p < .001$; ** $p < .01$; * $p < .05$; † $p < .10$

There were significant gains between T0 and T1, T0 and T2, T0 and T3, and T1 and T3. Between T2 and T3 there were marginal gains.

Intra-individual changes between T0 and T3 were also analyzed for weight, and in order to do that the differences between T0 and T3[2] were calculated for every child and then plotted as follows (Figure 7).

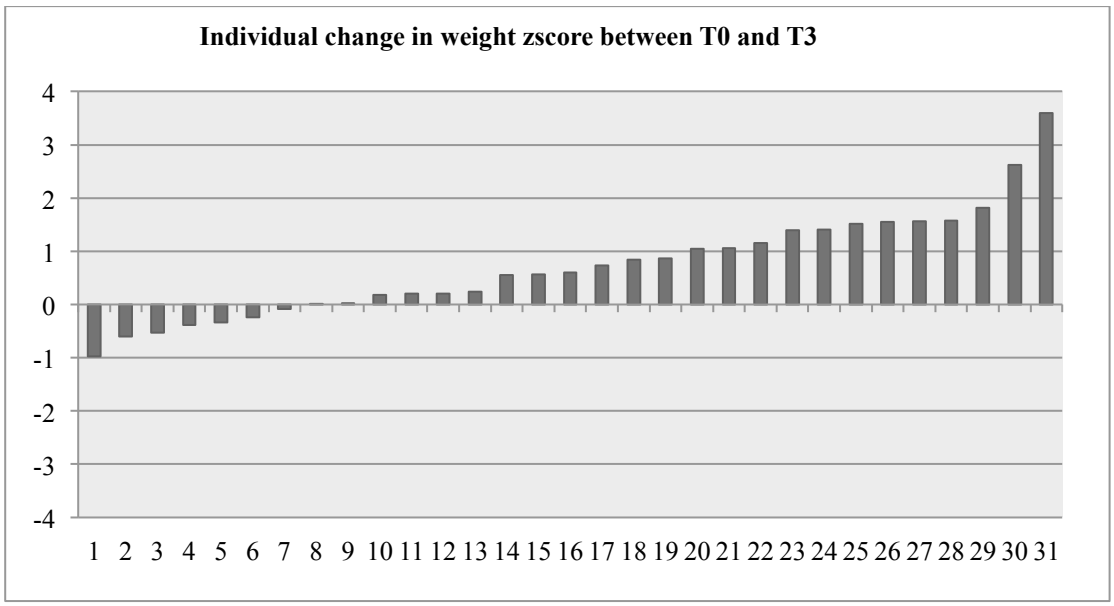

Figure 7: Intra-individual changes in weight z scores across time

Most of the sample (77.42%) presented gains in weight between T0 and T3, with only 7 children (22.58%) loosing weight during that time period. It is also possible to observe that the magnitude of gains and losses is different between different children.

Head Circumference

In figure 8 are presented the head circumference z scores for each moment of assessment.

[2] Difference between T0 and T3 was calculated as follows: Weight z score at T3 – Weight z score at T0. Positive differences mean that at T3 the weight z score values were higher than at T0. Negative differences mean the opposite.

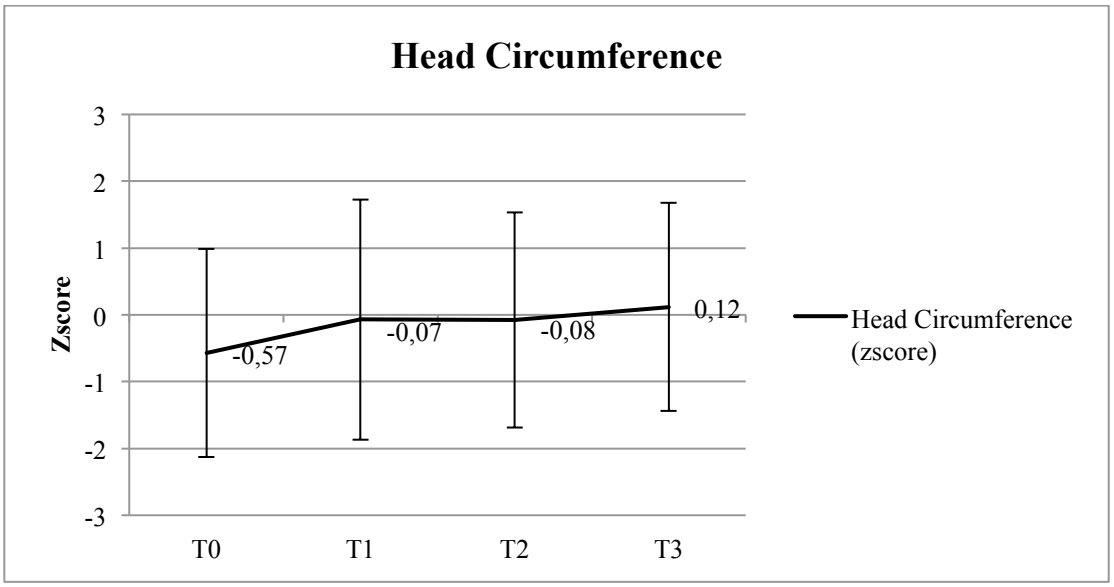

Figure 8: Evolution of child's head circumference z scores across time

At T0 the mean head circumference z score was -0.57 (*SD* = 1.56), at T1 the mean was -0.07 (*SD* = 1.80), at T2 it was -0.08 (*SD* = 1.61), and finally at T3 it was -0.12 (*SD* = 1.56). At T0 and T1 5 children (16.13%) were two standard deviations below the mean, while at T2 and T3 only 3 children (9.68%) were two standard deviations below the mean.

The effect of time revealed significant gains in terms of head circumference growth, across time spent in institution, χ^2 (3) = 14.34, p = .002.

The following table (Table 7) presents the pairwise comparisons between moments of assessment for head circumference z scores.

Table 7

Comparisons between moments of assessment for head circumference

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
Head Circumference (zscore)	*	**	**	*ns*	*ns*	†

** $p < .01$; * $p < .05$; † $p < .10$

There were gains between T0 and T1, T0 and T2, T0 and T3, and marginal gains between T2 and T3.

As was presented for the other domains of physical growth, intra-individual changes between T0 and T3[3] were also calculated for head circumference z scores, and plotted as follows (Figure 9).

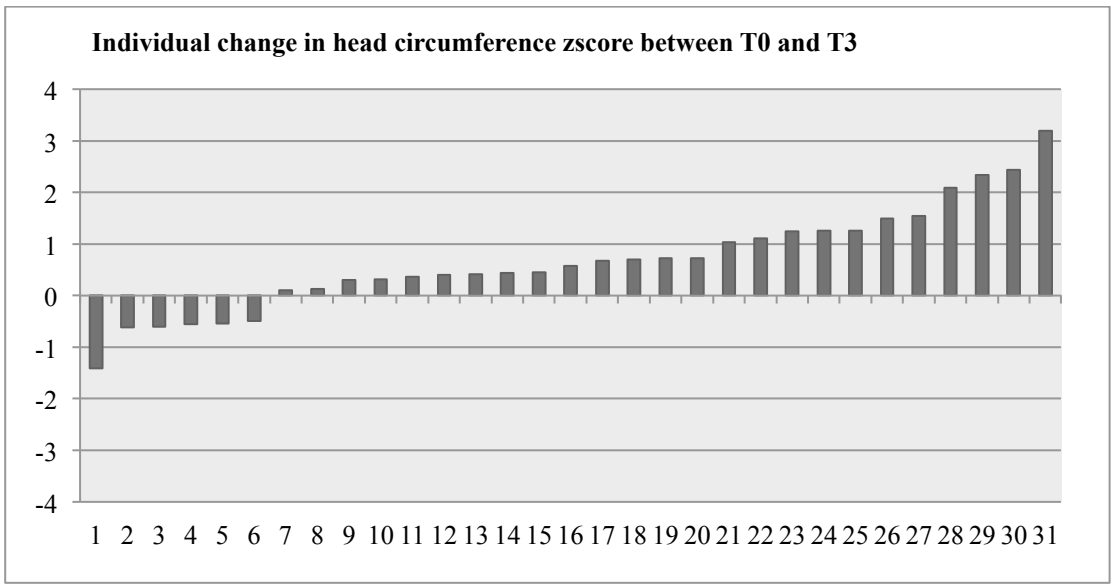

Figure 9: Intra-individual changes in head circumference z scores across time

As was the case for weight, also for head circumference most of the children (80.65%) revealed gains between T0 and T3, with only 6 children (19.35%) showing a decrease in their head circumference z score between T0 and T3. Also, the magnitude of the losses and gains were different from child to child.

[3] Difference between T0 and T3 was calculated as follows: Head circumference z score at T3 – Head circumference z score at T0. Positive differences mean that at T3 the head circumference z score values were higher than at T0. Negative differences mean the opposite.

1.2. Effect of age at admission in physical growth

The effect of age at admission was also tested, using a dichotomized age with the cutoff point at 12 months. The results are as follows in Figure 10 and Table 8.

Height

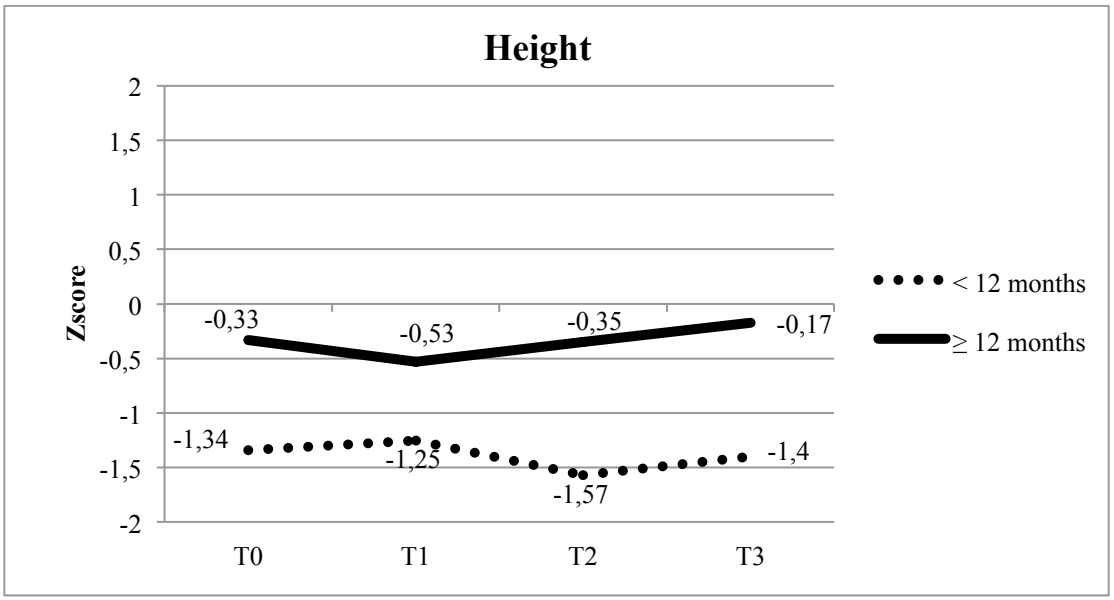

Figure 10: Height differences between the two age groups (below or above 12 months at admission) across time

Regarding the main effect of time tested for the groups separately, it was found that for the children admitted below 12 months there were no significant gains in height across time spent in the institution, χ^2 (3) = 6.13, p = .11, as well as for the children admitted above 12 months, χ^2 (3) = 3.73, p = .29. Nevertheless, it should be noted that for the children admitted earlier than 12 months a marginal significant loss occurred between T1 and T2 (Z = -1.68, p = .093), and for the children admitted above 12 months marginal significant increases in height occurred between T1 and T3 (Z = -1.68, p = .093). Regarding the differences between the two age groups (Table 8), they emerge only, and marginally, at T2, U = 59.00, p = .052, and at T3, U = 62.50, p = .072.

Table 8

Differences between age groups in height across time

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Height at T0	-1.34 (1.96)	-.33 (1.45)	73.00
Height at T1	-1.25 (1.67)	-.53 (1.42)	78.50
Height at T2	-1.57 (1.59)	-.35 (1.16)	59.00†
Height at T3	-1.40 (1.74)	-.17 (1.28)	62.50†

†$p < .10$.

Weight

In figure 11 is presented the evolution of weight growth across time, for the two age groups and the differences between them.

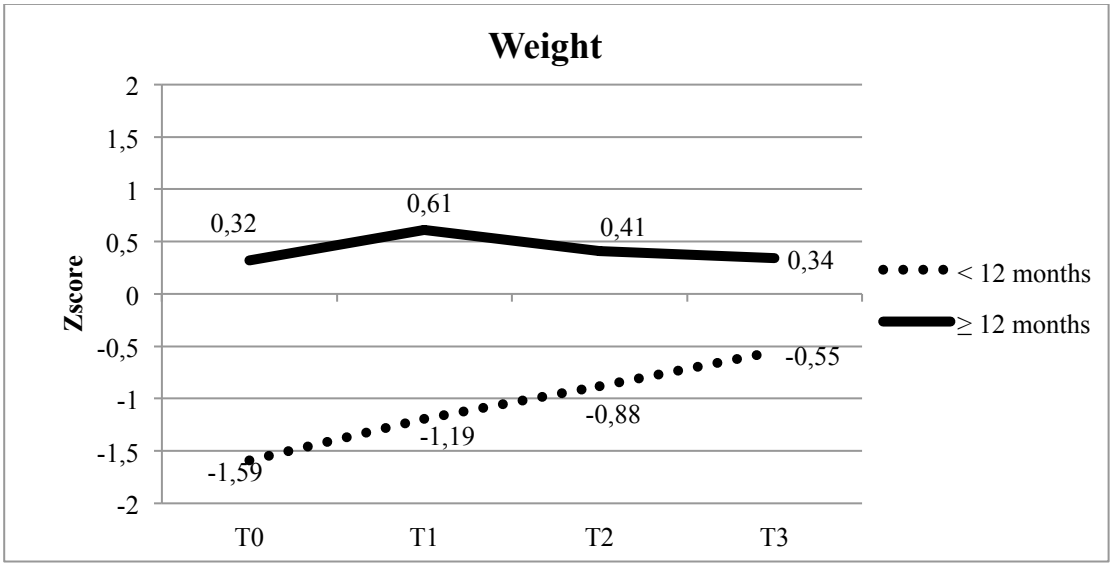

Figure 11: Weight differences between the two age groups (below or above 12 months at admission) across time

The analysis testing the main effect of time revealed that for children admitted below 12 months there were significant gains across time spent in the institution, χ^2 (3) = 24.03, p = .000, while for the children admitted above 12 months there were no significant gains across time spent in the institution, χ^2 (3) = 6.06, p = .11. Indeed, for the children admitted below 12 months, gains were noted between every adjacent and non-adjacent moments of assessment, with the only exception made between T1 and T2,

where no significant gain occurred. For the children admitted above 12 months, a significant loss of weight occurred between T1 and T3 (Z = -2,31, p = .021). Nevertheless, as depicted in Figure 11, children admitted above 12 months were always in a better physical condition, in terms of weight, across time spent in the institution. However, it should be noted that the significant differences between the two age groups tend to disappear throughout time, as can be seen in the table below (Table 9). At T2 differences are only marginally significant, and at T3 no significant differences were noted between the two age groups. Indeed, while for the group admitted below 12 months there was increase in weight across time, for the other group a tendency to decrease across time was verified.

Table 9

Differences between age groups in weight across time

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Weight at T0	-1.59 (1.65)	.32 (1.11)	36.00**
Weight at T1	-1.19 (1.64)	.61 (1.20)	31.50**
Weight at T2	-.88 (1.78)	.41 (1.19)	59.00†
Weight at T3	-.55 (1.79)	.34 (1.28)	70.50

**p< .01; †p< .10

Head Circumference

Analyses of the differences between the two age groups were also run for head circumference, as well as analysis of the evolution across time (Figure 12).

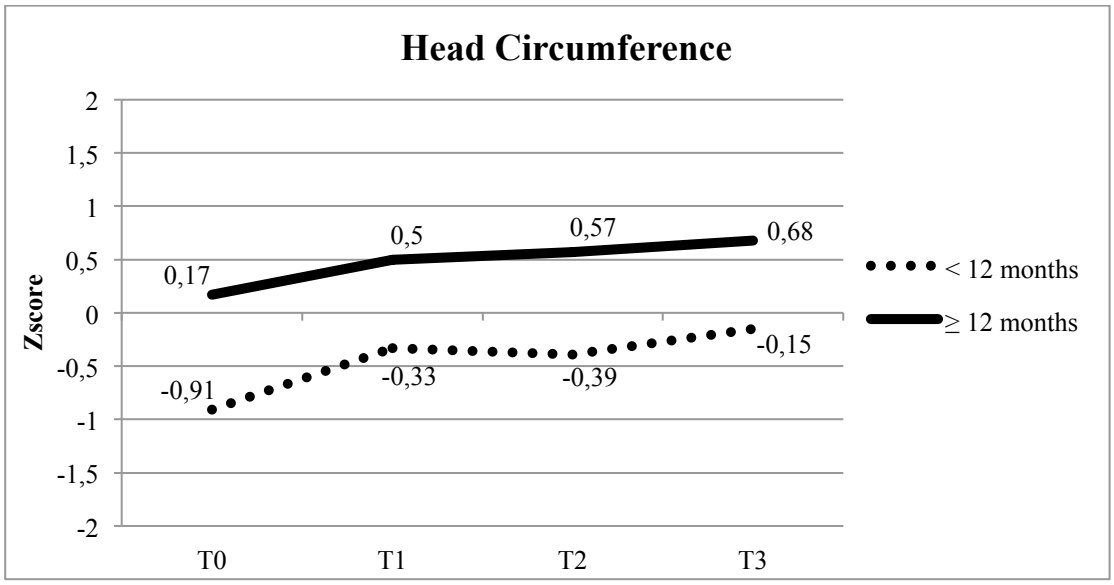

Figure 12: Head Circumference differences between the two age groups (below or above 12 months at admission) across time

It was found that for children admitted below 12 months there were significant gains across time spent in the institution, χ^2 (3) = 12.14, p = .007, that occurred mainly between T0 and T1 (Z = -2.29, p = .022), since no gains were noted between other adjacent moments. For children admitted above 12 months there was no significant change across time spent in the institution, χ^2 (3) = 2.82, p = .42, corroborated by no gains when pairwise comparisons were analyzed. No differences between groups were found for head circumference (Table 10).

Table 10

Differences between age groups in head circumference across time

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Head Circumference at T0	-.91 (1.51)	.17 (1.47)	72.50
Head Circumference at T1	-.33 (1.76)	.50 (1.84)	76.50
Head Circumference at T2	-.39 (1.55)	.57 (1.61)	70.00
Head Circumference at T3	-.15 (1.53)	.68 (1.57)	73.50

1.3. The role of the prenatal experiences in the child's physical growth from the time of admission at the institution (T0) onwards (T1, T2, and T3)

Association between Prenatal risk, Birth Weight and Physical growth at T0 (admission at the institution)

Table 11 shows that there were significant and negative correlations between prenatal risk and height z score (r_s = -.46, p = .012), and weight z score (r_s = -.55, p = .002), and head circumference z score (r_s = -.45, p = .014) at the admission at the institution. The results suggest that children who had experienced more risk during prenatal life were more likely to show poor physical growth in terms of height, weight, and head circumference at the admission at the institution. Birth weight was also taken as a proxy measure of the risk experienced during prenatal life, which was not included in the prenatal risk composite measure. As showed in Table XX, birth weight was positively and significantly associated with height z score (r_s = .74, p = .000), weight z score (r_s = .58, p = .000) and head circumference z score (r_s = .63, p = .001), suggesting that children with a better physical condition at birth were more likely to show a better physical condition at the admission at the institution, in terms of height, weight and head circumference.

Table 11

Correlations between prenatal risk and birth weight and child's physical growth at T0

	Height	Weight	Head Circumference
Prenatal Risk (n = 29)	-.46*	-.55**	-.45*
Birth Weight (n = 23)	.74**	.58**	.63**

Note. Spearman correlations; *** p< .001; **p< .01; *p< .05

Association between Prenatal risk, Birth Weight and Physical growth at T1

At T1 Spearman correlations revealed negative and significant associations between prenatal risk experienced and physical growth in terms of height z score (r_s = -.49, p = .007) and weight z score (r_s = -.50, p = .006), and marginally significant associations between prenatal risk and head circumference z score (r_s = -.37, p = .051)

(Table 12), meaning that children who experienced more risk in prenatal life tended to have lower height, weight and head circumference after living approximately 2 months in the institution. Results regarding associations with birth weight, also displayed in Table 12, revealed that birth weight was significantly associated with all the three domains of physical growth at T1, height (r_s = .73, p = .000), weight (r_s = .51, p = .012), and head circumference (r_s = .70, p = .000), suggesting that children who were born weighing more were more likely to show better physical growth outcomes at T1.

Table 12

Correlations between prenatal risk and birth weight and child's physical growth at T1

	Height	Weight	Head Circumference
Prenatal Risk (n = 29)	-.49**	-.50**	-.37†
Birth Weight (n = 23)	.73***	.51*	.70***

Note. Spearman correlations; *** p< .001; **p< .01; *p< .05

Association between Prenatal risk, Birth Weight and Physical growth at T2

Table 13 presents the associations between prenatal risk and physical growth at T2, for which there were negative and marginally significant associations with height z score (r_s = -.36, p = .056) and weight z score (r_s = -.36, p = .053). Indeed, it seems that from T0 to T2 the associations between prenatal risk and physical growth have been weakening. By contrast, there were significant and positive associations between birth weight and height z score (r_s = .68, p = .000), weight z score (r_s = .51, p = .014), and head circumference (r_s = .77, p = .000) at T2. Again, children who presented higher weight at birth were more likely to show better physical status while still living in the institution.

Table 13

Correlations between prenatal risk and birth weight and child's physical growth at T2

	Height	Weight	Head Circumference
Prenatal Risk (n = 29)	-.36†	-.36†	-.26
Birth Weight (n = 23)	.68***	.51*	.77***

Note. Spearman correlations; *** p< .001; **p< .01; *p< .05 †p< .10.

Association between Prenatal risk, Birth Weight and Physical growth at T3

Spearman correlations revealed a negative and marginally significant association between prenatal risk and child's height at T3 (r_s = -.34, p = .069), approximately 8 months after the admission at the institution, and negative and significant associations between prenatal risk and child's weight (r_s = -.45, p = .014) and head circumference (r_s = -.39, p = .039), as reported in Table 14. Regarding associations between birth weight and physical growth it were significant for height (r_s = .58, p = .004), weight (r_s = .62, p = .002), and head circumference (r_s = .73, p = .000), meaning that the higher the weight at birth, the higher the z scores on each of the physical domains assessed.

Table 14

Correlations between prenatal risk and birth weight and child's physical growth at T3

	Height	Weight	Head Circumference
Prenatal Risk (n = 29)	-.34†	-.45*	-.39*
Birth Weight (n = 23)	.58**	.62**	.73***

Note. Spearman correlations; **p< .01; *p< .05; †p< .10.

1.4. The role of the prenatal experiences in the child's gain/loss in physical growth between T0 and T3

Spearman correlations (Table 15) were only significant for the association between birth weight and height differences between T0 and T3, suggesting that higher gains in height during the time spent in the institution were associated with less birth height (r_s= -.51, p = .012).

Table 15

Correlations between prenatal risk and birth weight and height, weight and head circumference differences between T0 and T3

	Height differences between T0 and T3	Weight differences between T0 and T3	Head Circumference differences between T0 and T3
Prenatal Risk (n = 29)	.14	.17	.20
Birth Weight (n = 23)	-.51*	-.07	.009

Note. Spearman correlations; *$p < .05$

Taking into account the results presented above it was interesting at this point to analyze the difference between the two age groups (below or above 12 months at admission) in terms of their birth weight and the prenatal risk experienced. Statistical analysis revealed no differences between the two groups in terms of birth weight (U = 72.50, $p = .701$) and prenatal risk experienced (U = 50.00, $p = .103$).

1.5. The role of the early family context risks in the child's physical growth from the time of admission at the institution (T0) onwards (T1, T2, and T3)

Association between Family-relational risk, Emotional-negligence risk and Physical growth at T0 (admission at the institution)

In Table 16 are listed the values of Spearman correlations between the variables presented. Results revealed no significant associations between family-relational risk experienced before institutionalization and growth at the admission at the institution, as well as between emotional-negligence risk experienced and growth at the admission time.

Table 16

Correlations between Family-relational risk, Emotional-negligence risk and child's physical growth at T0

	Height	Weight	Head Circumference
Family-Relational Risk (n = 29)	-.03	.06	.03
Emotional-Negligence Risk (n = 31)	-.06	.20	.03

Note. Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and Physical growth at T1

The following table (Table 17) shows no significant associations between family-relational risk, emotional-negligence risk and z scores of height, weight, and head circumference at T1.

Table 17

Correlations between Family-relational risk, Emotional-negligence risk and child's physical growth at T1

	Height	Weight	Head Circumference
Family-Relational Risk (n = 29)	.17	.12	.31
Emotional-Negligence Risk (n = 31)	.06	.23	.08

Note. Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and Physical growth at T2

Also at T2 there were no significant associations between the risk experienced while living with the biological family, in terms of relational risk and emotional-negligence risk, and child's growth while living in the institution (Table 18).

Table 18

Correlations between Family-relational risk, Emotional-negligence risk and child's physical growth at T2

	Height	Weight	Head Circumference
Family-Relational Risk (n = 29)	.02	.24	.23
Emotional-Negligence Risk (n = 31)	.10	.26	.14

Note. Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and Physical growth at T3

At T3, approximately 8 months after the child's admission at the institution, there were no associations between the family-relational risk and emotional-negligence risk previously experienced and child's physical growth in terms of height, weight, and head circumference (Table 19).

Table 19

Correlations between Family-relational risk, Emotional-negligence risk and child's physical growth at T3

	Height	Weight	Head Circumference
Family-Relational Risk (n = 29)	.06	.17	.29
Emotional-Negligence Risk (n = 31)	.11	.13	-.01

Note. Spearman correlations

1.6. The role of the early family context in the child's gain/loss in physical growth between T0 and T3

Spearman correlations revealed no associations between risk experienced in the early family context and the child's gains/losses in physical growth (z scores) during the period between T0 and T3 in the institution (Table 20).

Table 20

Correlations between Family-relational risk, Emotional-negligence risk and height, weight and head circumference differences between T0 and T3

	Height differences between T0 and T3	Weight differences between T0 and T3	Head Circumference differences between T0 and T3
Family-Relational Risk (n = 29)	.19	.000	.22
Emotional-Negligence Risk (n = 31)	.25	-.26	-.02

Note. Spearman correlations

Considering the results presented above it revealed interesting at this point to examine the difference between the two age groups (below or above 12 months at admission) in terms of the family-relational risk and emotional-negligence experienced. Statistical analysis revealed no differences between the two groups in terms of family-relational risk (U = 83.00, p = .764) and emotional-negligence risk experienced (U = 73.00, p = .186).

1.7. The role of the child's status at the admission at the institution in the child's physical growth during institutionalization

Association between Height at admission and Height at T1, T2 and T3

Table 21 report associations between the child's physical status at the admission, in terms of height at admission, and the height growth at T1, T2, and T3. Associations between height at T0 and height at T1 (r_s = .77, p = .000), T2 (r_s = .78, p = .000) and T3 (r_s = .84, p = .000) were all positive and significant, meaning that higher height at the admission at the institution was associated with better height growth during the institutionalization period.

Table 21

Correlations between Height at admission and Height at T1, T2 and T3

	Height T0	Height T1	Height T2	Height T3
Height at T0 (admission) (n = 31)	-	.77***	.78***	.84***

Note. Spearman correlations; ***p< .001; $^{\dagger}p$< .10.

Association between Weight at admission and Weight at T1, T2 and T3

The child's status at the admission in terms of weight at admission was also associated with the child's weight at T1, T2 and T3 (Table 22). Weight at the admission revealed to be significant and positively associated with weight at T1 (r_s = .86, p = .000), at T2 (r_s = .73, p = .000), and at T3 (r_s = .71, p = .000), meaning that the higher weight when entering the institution the higher the weight in the subsequent months.

Table 22

Correlations between Weight at admission and Weight at T1, T2 and T3

	Weight T0	Weight T1	Weight T2	Weight T3
Weight at T0 (admission) (n = 31)	-	.86***	.73***	.71***

Note. Spearman correlations; ***p< .001; **p< .01; $^{\dagger}p$< .10.

Association between Head Circumference at admission and Head Circumference at T1, T2 and T3

Spearman correlations (Table 23) revealed significant and positive associations between child's status at the admission in terms of head circumference and the subsequent z scores of head circumference growth at T1 (r_s= .81, p = .000), T2 (r_s= .79, p = .000) and T3 (r_s= .71, p = .000).

Table 23

Correlations between Head Circumference at admission and Head Circumference at T1, T2 and T3

	Head Circumference T0	Head Circumference T1	Head Circumference T2	Head Circumference T3
Head Circumference at T0 (admission) (n = 31)	-	.81***	.79***	.71***

Note. Spearman correlations; ***p< .001; $^{\dagger}p$< .10.

1.8. The role of the child's status at the admission at the institution in the child's gain/loss in physical growth between T0 and T3

Association between the child's physical status at the admission and physical gain/loss between T0 and T3 were also tested (Table 24).

Table 24

Correlations between Height, Weight, Head Circumference at admission and height, weight and head circumference differences between T0 and T3

	Height differences between T0 and T3	Weight differences between T0 and T3	Head Circumference differences between T0 and T3
Height at T0 (admission) (n = 31)	-.40*	-	-
Weight at T0 (admission) (n = 31)	-	-.44*	-
Head Circumference at T0 (admission) (n = 29)	-	-	-.36*

Note. Spearman correlations; **p< .01; *p< .05

Results presented in Table 24 revealed that higher gains in height between T0 and T3 were associated with less height at the admission at the institution (r_s= -.40, p = .024), higher gains in weight between T0 and T3 were associated with less weight at the institutional placement (r_s= -.44, p = .013), and higher gains in head circumference

between T0 and T3 were associated with less head circumference at the admission moment (r_s= -.36, p = .049). This pattern of results suggests that children who gained more height, weight and head circumference between T0 and T3 were the ones that were admitted in the poorest condition in terms of height, weight and head circumference. The opposite is also true, children admitted in better physical conditions were more likely to be the ones who loose or gain less height, weight and head circumference throughout institutionalization.

1.9. The role of the quality of the institutional context in the child's physical growth during institutionalization (T1, T2, and T3)

Associations between the quality of the institutional context in terms of human resources, equipment and material resources, basic needs routines, developmental activities and caregiving stability, and child's physical growth were only conducted for T1, T2 and T3, since at T0 the child's physical status is thought to reflect his/her life history in early family context, much more than a reflex of the institutional care.

Association between institutional context variables and height, weight and head circumference at T1

Table 25 presents the association between child's physical growth at T1 and the variables of the quality of the institutional caregiving context. Spearman correlations revealed no significant associations between the variables presented.

Table 25

Correlations between physical growth at T1 and institutional quality context

	Height	**Weight**	**Head Circumference**
Human Resources	.02	-.05	-.03
Equipment and material resources	.13	.20	-.01
Basic Needs Routines	.24	.15	.16
Developmental activities	.15	.19	.17
Caregiving stability	-.24	-.06	-.22

Note. Spearman correlations

Association between institutional context variables and height, weight and head circumference at T2

Similarly to the results presented above, there were no significant associations between variables of the quality of the institutional context and child's physical status at T2 (Table 26).

Table 26

Correlations between physical growth at T2 and institutional quality context

	Height	**Weight**	**Head Circumference**
Human Resources	-.17	-.28	-.10
Equipment and material resources	.29	.02	.11
Basic Needs Routines	.20	.14	.09
Developmental activities	.15	-.04	.25
Caregiving stability	-.31	-.10	-.18

Note. Spearman correlations

Association between institutional context variables and height, weight and head circumference at T3

Also, at T3 Spearman correlations revealed no significant associations between the quality of the institutional context and child's physical growth (Table 27).

Table 27

Correlations between physical growth at T3 and institutional quality context

	Height	**Weight**	**Head Circumference**
Human Resources	.05	-.25	-.10
Equipment and material resources	.29	-.03	.09
Basic Needs Routines	.22	.07	.02
Developmental activities	.23	-.10	.23
Caregiving stability	-.11	-.15	-.18

Note. Spearman correlations

1.10. The role of the quality of institutional context in the child's gain/loss in physical growth between T0 and T3

Association between the quality of the institutional context and physical gain/loss between T0 and T3 were also tested (Table 28).

Results revealed a single significant association between one of the sub-dimensions of institutional quality context and the size of gain/loss in physical growth between T0 and T3 (r_s= -.41, p = .009), meaning that institutions where there were more human resources tended to provide higher gains in child's height between T0 and T3

Table 28

Correlations between institutional quality context and height, weight and head circumference differences between T0 and T3

	Height differences between T0 and T3	Weight differences between T0 and T3	Head circumference differences between T0 and T3
Human Resources	.41**	-.17	-.15
Equipment and material resources	.14	-.20	-.09
Basic Needs Routines	-.13	-.09	-.12
Developmental activities	.07	-.26	-.01
Caregiving stability	.28	.07	.16

Note. Spearman correlations; **p< .01

Again, at this point it was interesting to analyze the differences between the two age groups (below or above 12 months) in terms of the quality of the institutional care they received. Statistical analysis revealed significant differences in terms of the access to equipment and material resources (U = 53.00, p = .028) and in terms of the developmental activities promoted (U = 53.50, p = .028). Older children at admission had access to more equipment and material (e.g., toys and equipment that promote development) and were also in institutions where more developmental activities were promoted by the technical and caregiver's staff.

1.11. The role of the caregiver's sensitivity and cooperation behaviors in the child's physical growth during institutionalization (T1, T2, and T3)

Association between caregiver's sensitivity and cooperation in play interaction with the child and height, weight and head circumference at T1

As it was the case for the associations between quality of institutional care and physical growth, also for caregiver's sensitivity and cooperation the analyses were only

conducted from T1 onwards, for the same reasons outlined above. Spearman correlations revealed no significant associations between either caregiver's sensitivity and cooperation in interaction with the child and his/her physical growth at T1 (Table 29).

Table 29

Correlations between caregiver's sensitivity and cooperation and physical growth at T1

	Height	Weight	Head Circumference
Sensitivity (n = 28)	-.17	-.08	-.17
Cooperation (n = 28)	-.21	-.01	-.09

Note. Spearman correlations

Association between caregiver's sensitivity and cooperation in play interaction with the child and height, weight and head circumference at T2

Also at T2, approximately 5 months after admission, there were no significant associations between the child's physical growth and the caregiver's sensitivity and cooperation behaviors in interaction with the child (Table 30).

Table 30

Correlations between caregiver's sensitivity and cooperation and physical growth at T2

	Height	Weight	Head Circumference
Sensitivity (n = 31)	-.09	.07	-.21
Cooperation (n = 30)	-.17	-.003	-.18

Note. Spearman correlations

Association between caregiver's sensitivity and cooperation in play interaction with the child and height, weight and head circumference at T3

As it was the case for previous moments of assessment, also at T3 there were no significant associations between caregiver's sensitivity and cooperation behaviors towards children and their physical growth throughout institutionalization (Table 31).

Table 31

Correlations between caregiver's sensitivity and cooperation and physical growth at T3

	Height	**Weight**	**Head Circumference**
Sensitivity (n = 30)	.14	.18	.07
Cooperation (n = 30)	.000	.04	-.06

Note. Spearman correlations

1.12. The role of the caregiver's sensitivity and cooperation behaviors in the child's gain/loss in physical growth between T0 and T3

To perform correlations between the gain/loss in physical growth between T0 and T3 and caregiver's sensitivity and cooperation behaviors, the measurement of the caregiver's sensitivity and cooperation at T0 was included in the analysis since the sensitivity and cooperation experienced at T0 can contribute to the differences in physical growth occurring from T0 to T3. The measurement at T3 was excluded since it was not expected to be associated with the physical gain/loss until T3.

Spearman correlations revealed a single significant association between caregiver's sensitivity behaviors at T2 and the size of gain/loss in head circumference growth between T0 and T3 (r_s= .37, p = .040), suggesting that children that experienced higher levels of caregiver's sensitivity at T2 tended to show higher gains in head circumference growth during the period of institutionalization (Table 32).

Table 32

Correlations between caregiver's sensitivity and cooperation and height, weight and head circumference differences between T0 and T3

	Height differences between T0 and T3	Weight differences between T0 and T3	Head Circumference differences between T0 and T3
Sensitivity T0 (n=25)	.10	.03	.16
Cooperation T0 (n=25)	-.02	.10	.10
Sensitivity T1 (n=28)	.01	-.16	.15
Cooperation T1 (n=28)	-.09	-.19	-.04
Sensitivity T2 (n=31)	.29	-.24	.37*
Cooperation T2 (n=30)	.13	-.20	.03

Note. Spearman correlations

At this point it was interesting to understand the differences between the two age groups in terms of the caregiving sensitive and cooperative behaviors they experienced. Older children at admission (above or equal to 12 months) experienced more sensitivity by their caregivers at T1 (U = 45.50, p = .079) and at T3 (U = 39.50, p = .006), and also more cooperative behaviors at T3 (U = 38.00, p = .005), than children admitted earlier (below 12 months).

2. MENTAL DEVELOPMENT

Our second domain of interest in the developmental trajectories of institutionalized child concerns mental development in terms of their cognitive, language and motor progress.

2.1. Evolution across time for cognitive, language and motor development

Thus, we were interested in understanding the child's mental development throughout the time spent in the institution. In this sense, we aim to assess the groups' evolution across time in cognitive, language and motor domain. The results are as follows.

Cognitive development

In figure 13 are presented the percentiles for cognitive development in each moment of assessment.

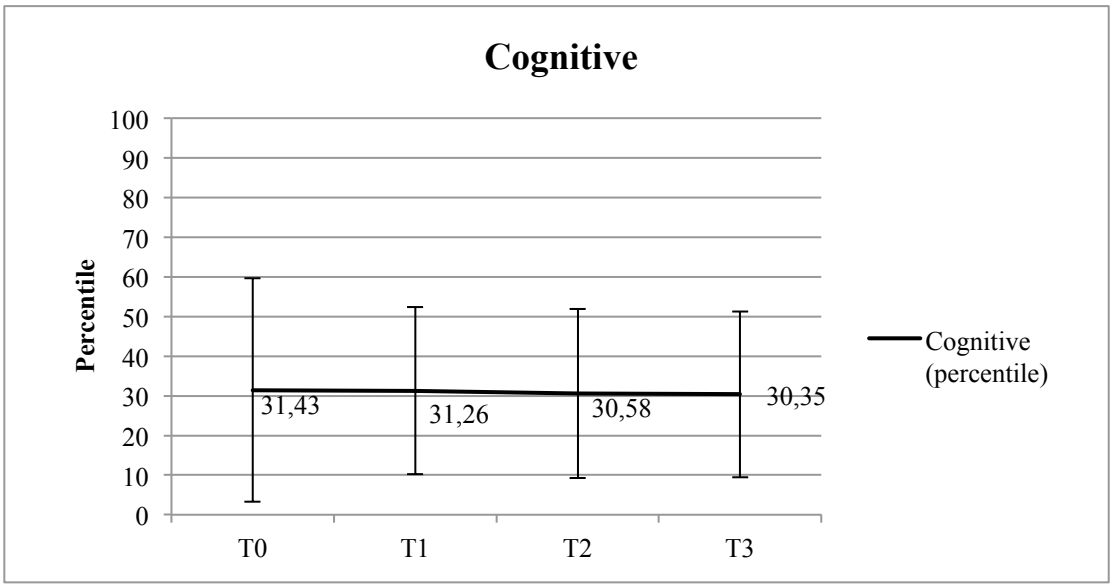

Figure 13: Child's cognitive evolution across time

At T0 the mean percentile was 31.43 (SD = 28.19), at T1 it was 31.26 (SD = 21.06), at T2 it was 30.58 (SD = 21.38), and finally at T3 it was 30.35 (SD = 20.90). When the main effect of time for cognitive development was analyzed, results showed that there was no gain across time spent in institution, F (3, 90) = .02, p = .995.

Intra-individual changes were also analyzed. Calculation of the differences between T0 and T3[4] for every child were conducted and then plotted as follows. Figure 14 shows the wide variability in the magnitude of losses and gains across time spent in the institution. 13 children (41.94%) presented losses in their cognitive development between T0 and T3, while 18 children (58.06%) presented gains in their cognitive competence across time spent in the institution. This means that for 13 children their cognitive competence at the institutional placement (T0) were better than approximately 8 months later (T3), while for 18 children gains across time in terms of their cognitive development were registered.

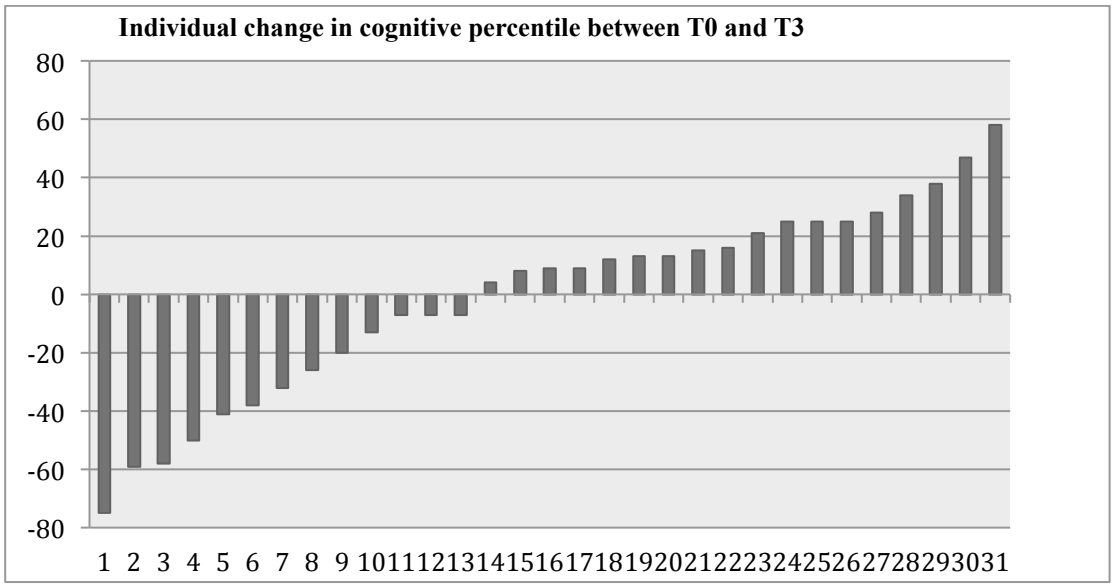

Figure 14: Intra-individual changes in cognitive percentiles across time

Language development

In figure 15 are presented the language percentiles for each moment of assessment.

[4] Difference between T0 and T3 was calculated as follows: Cognitive percentile at T3 – Cognitive percentile at T0. Positive differences mean that at T3 the cognitive percentile were higher than at T0. Negative differences mean the opposite.

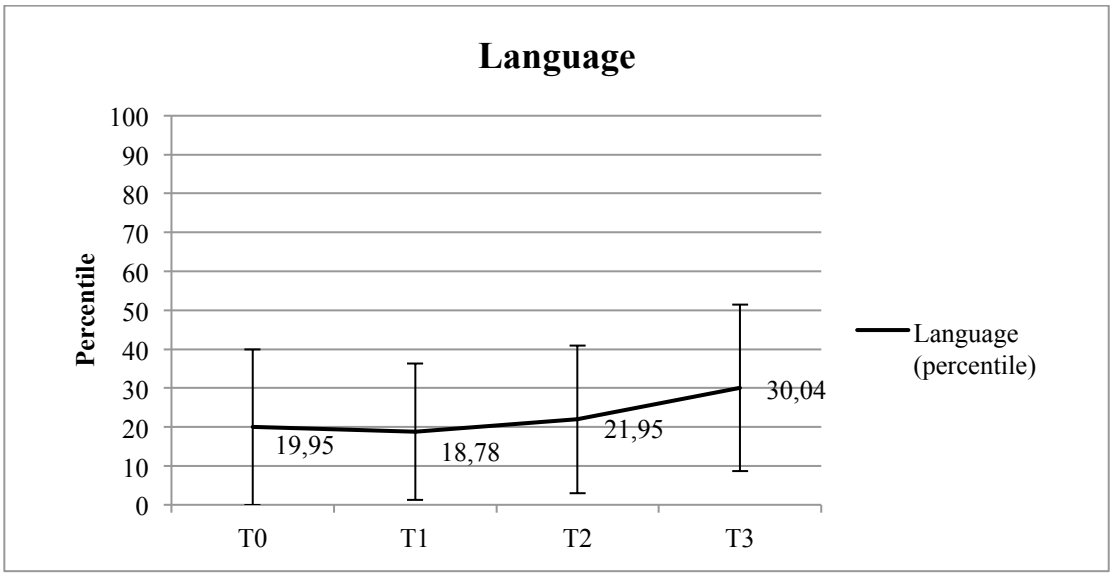

Figure 15: Child's language evolution across time

The mean percentile at T0 was 19.95 (*SD* = 20.00), at T1 it was 18.78 (*SD* = 17.52), at T2 it was 21.95 (*SD* = 18.97), while at T3 the mean percentile was 30.04 (*SD* = 21.43). Statistical analysis revealed significant gains in language development across time spent in institution, χ^2 (3) = 13.98, p = .003.

Table 33 presents the comparisons between moments of assessment for language development.

Table 33

Comparisons between moments of assessment for language development

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
Language (percentile)	*ns*	*ns*	**	*ns*	**	*

*** $p < .001$; ** $p < .01$; * $p < .05$; † $p < .10$

Also for language development were calculated the differences between T0 and T3[5] for every child and plotted the intra-individual changes. Figure 16 shows the wide variability in the magnitude of losses and gains across time spent in the institution. Only seven children (22.58%) presented losses in their language competence between T0 and T3. Three children (9.68%) remained at the same percentile at T0 and T3. On the other hand 21 children (67.74%) presented gains in their language competence between T0 and T3. Data suggests that there were more children increasing their language level than children loosing across time.

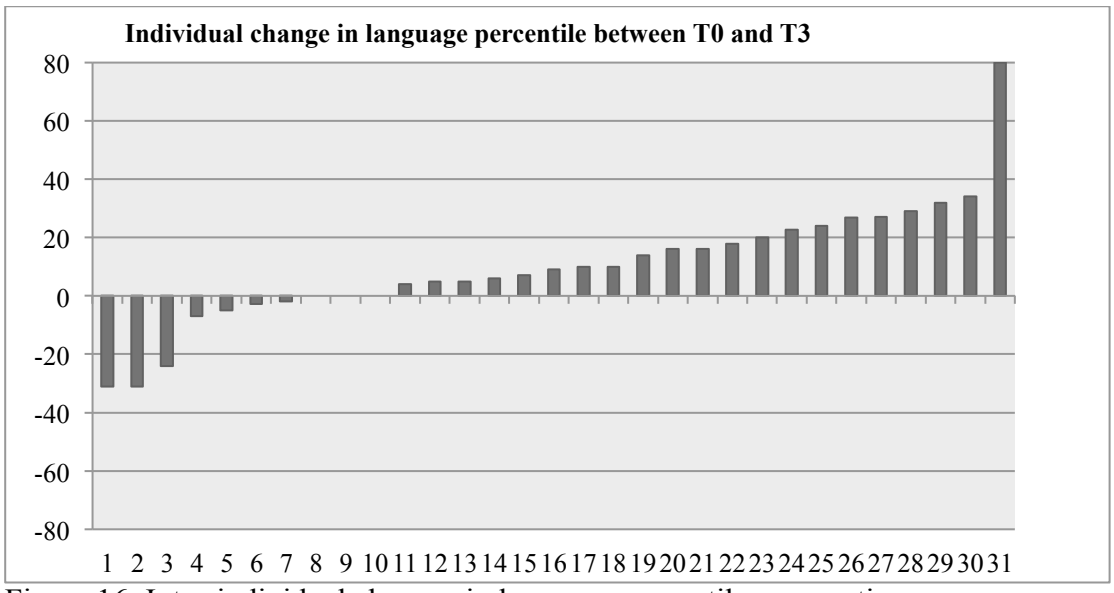

Figure 16: Intra-individual changes in language percentiles across time

Motor development

Motor percentiles for each moment of assessment are presented in Figure 17.

[5] Difference between T0 and T3 was calculated as follows: Language percentile at T3 – Language percentile at T0. Positive differences mean that at T3 the language percentile were higher than at T0. Negative differences mean the opposite.

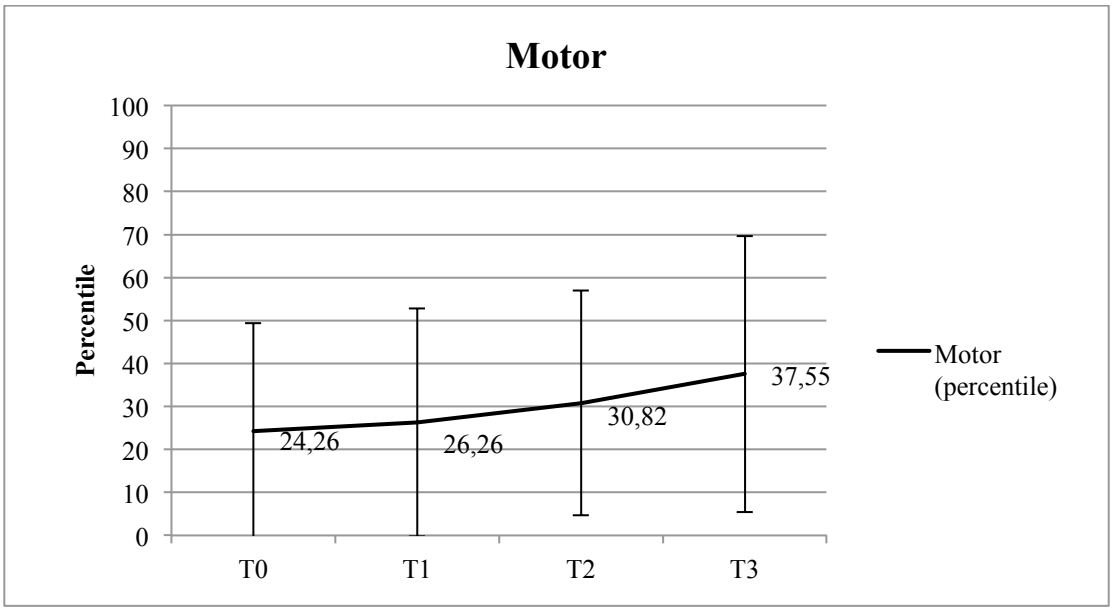

Figure 17: Child's motor evolution across time

At the institutional placement (T0) the mean percentile was 24.26 (*SD* = 25.11), at T1 it was 26.26 (*SD* = 26.48), at T2 it was 30.82 (*SD* = 26.17), and finally at T3 it was 37.55 (*SD* = 32.15). There were gains in terms of motor development, across time spent in institution, χ^2 (3) = 10.58, *p* = .014.

Table 34 presents the comparisons between moments of assessment for percentiles of motor development.

Table 34

Comparisons between moments of assessment for motor development

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
Motor (percentile)	*ns*	†	*	†	**	*ns*

*** $p < .001$; ** $p < .01$; * $p < .05$; † $p < .10$

The graph that follows (Figure 18) represents the intra-individual change in motor development between T0 and T3 for each child assessed. The differences between T0 and T3[6] were calculated for every child and then plotted.

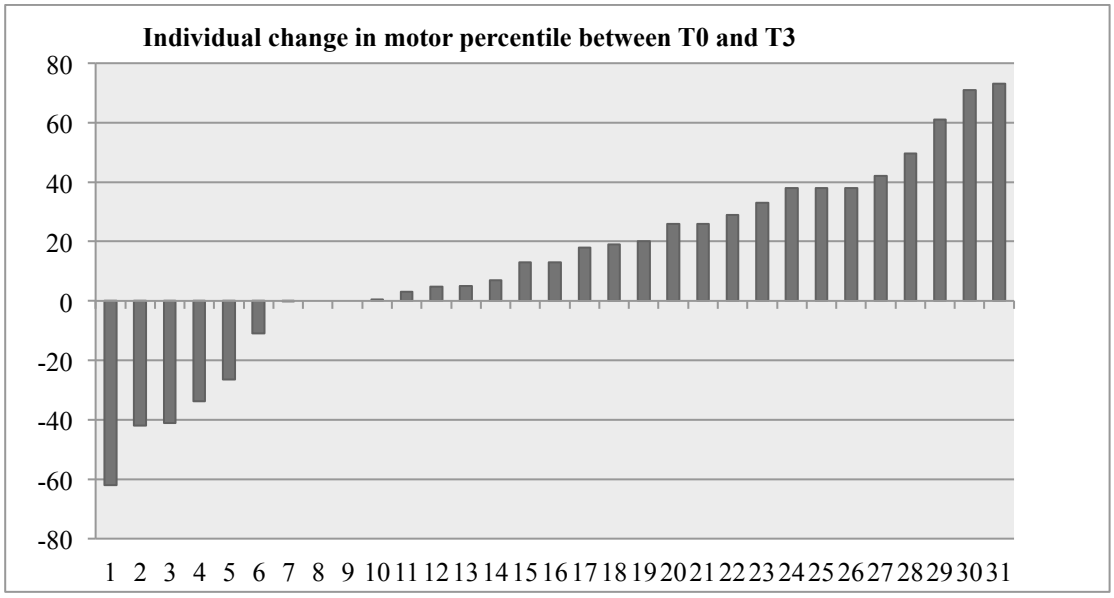

Figure 18: Intra-individual changes in motor percentiles across time

Seven children (22.58%) presented losses in their motor development between T0 and T3. Two children (6.45%) did not show either gains or losses between T0 and T3, while 22 children (70.97%) increased their motor competence from the admission at the institution until T3. Results suggested that there were more children who gained while institutionalized, than children loosing across time.

2.2. Effect of age at admission in mental development

The effect of age at admission was also tested for mental development using the dichotomized age with the cutoff point at 12 months. The results are as follows in Figure 19 and Table 35.

[6] Difference between T0 and T3 was calculated as follows: Motor percentile at T3 – Motor percentile at T0. Positive differences mean that at T3 the motor percentile were higher than at T0. Negative differences mean the opposite.

Cognitive

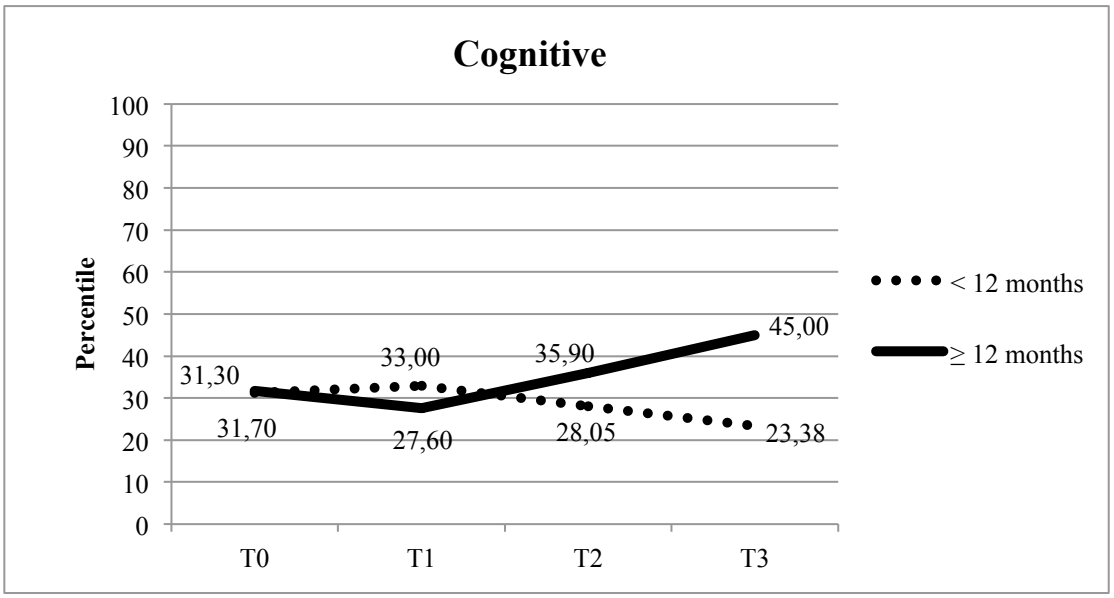

Figure 19: Cognitive differences between the two age groups (below or above 12 months at admission) across time

Analysis of the evolution across time for the age group admitted below 12 months revealed that there was no significant change across time spent in the institution, χ^2 (3) = 2.45, p = .49. The same was true for the group admitted above 12 months, for whom there was also no significant change throughout time, χ^2 (3) = 4.24, p = .24. Nevertheless, pairwise comparisons revealed a significant decrease in cognitive percentiles between T1 and T3 (Z = -2.05, p = .040) for the children admitted below 12 months, while for the children admitted above 12 months a significant gain occurred between T1 and T3 (Z = -2.14, p = .033).

In terms of the differences between groups in each moment of assessment, analyses of the differences revealed a single significant difference between the groups at T3, almost 8 months after children's institutional placement (Table 35). This means that children who were admitted below 12 months were at significant low levels of cognitive development at T3, when compared to children admitted above 12 months U = 44.50, p = .009.

Table 35

Differences between age groups in cognitive development

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Cognitive at T0	31.30 (29.90)	31.70 (25.74)	100.00
Cognitive at T1	33.00 (22.44)	27.60 (18.39)	94.50
Cognitive at T2	28.05 (22.14)	35.90 (19.70)	78.00
Cognitive at T3	23.38 (16.90)	45.00 (21.67)	44.50**

***p*< .01

Language

Analyses of the differences between the two age groups were conducted as well as analyses of the evolution across time for each age group. Results are as follows (Figure 20).

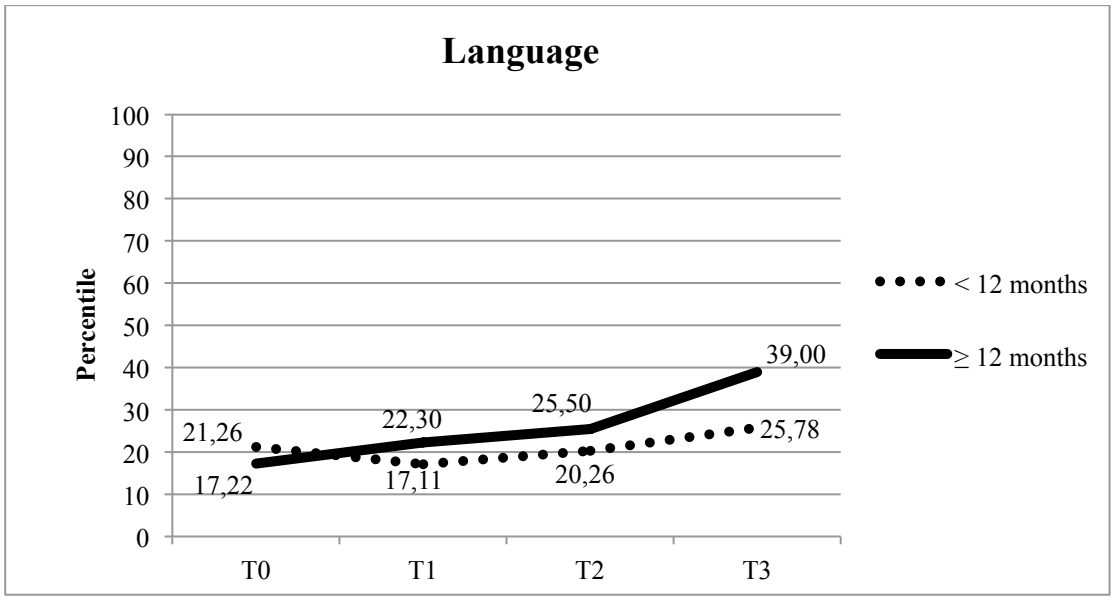

Figure 20: Language differences between the two age groups (below or above 12 months at admission) across time

Children admitted at the institution below 12 months showed marginally significant gains in language competence across time spent in the institution, χ^2 (3) = 6.44, p = .09, which occurred only between T1 and T3 (Z = -2.05, p = .040). For the children admitted above 12 months there were significant gains across time spent in the institution, χ^2 (3) = 12.54, p = .006, between T0 and T3 (Z = -2.50, p = .013), T1 and T3 (Z = -2.20, p = .028), and also between T2 and T3 (Z = -2.81, p = .005). Analyses of the

differences between the groups revealed no significant differences between them (Table 36).

Table 36

Differences between age groups in language development

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Language at T0	21.26 (21.49)	17.22 (17.14)	98.00
Language at T1	17.11 (17.45)	22.30 (18.04)	81.50
Language at T2	20.26 (18.53)	25.50 (20.38)	85.00
Language at T3	25.78 (16.44)	39.00 (28.27)	79.50

Motor

The same analyses were run for motor development and results are the following (Figure 21).

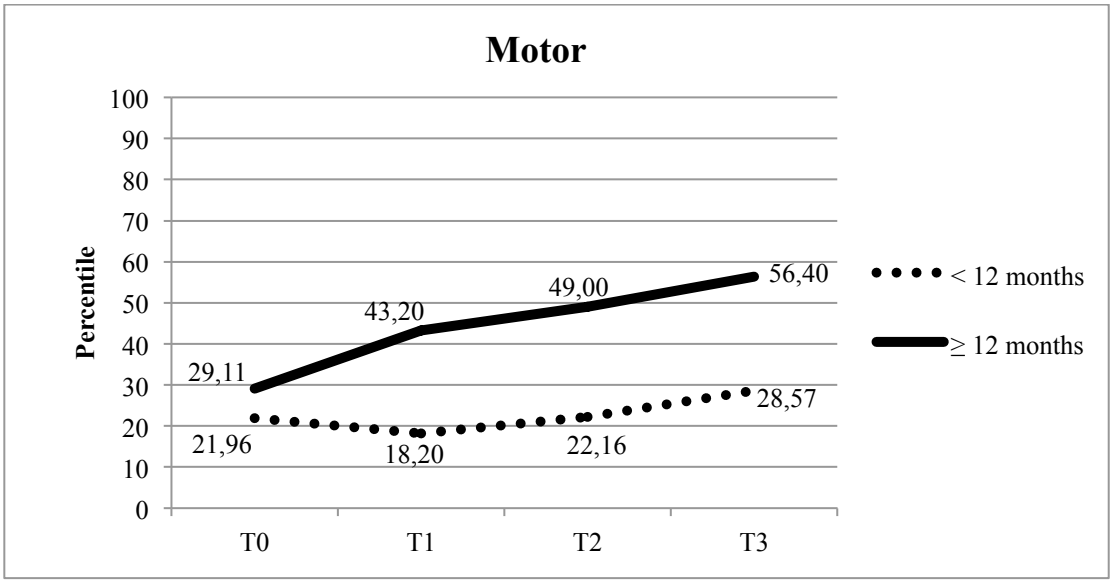

Figure 21: Motor differences between the two age groups (below or above 12 months at admission) across time

There was no significant change across time spent in the institution for the children admitted below 12 months, χ^2 (3) = 4.72, p = .19, despite the marginally significant gain between T1 and T3, (Z = -1.83, p = .067). By contrast there were significant gains across time spent in the institution for the children entering the

institution above 12 months, χ^2 (3) = 11.22, p = .011, occuring between T0 and T1 (Z = -1.84, p = .065), between T0 and T2 (Z = -2.31, p = .021), between T0 and T3 (Z = -2.81, p = .005), and also between T1 and T3 (Z = -2.09, p = .037).

Indeed, as presented in Table 37, there were significant differences between the two groups, wherein children admitted above 12 months showed higher motor functioning at T1, U = 51.50, p = .023, T2, U = 40.0, p = .006, and T3, U = 56.00, p = .038.

Table 37

Differences between age groups in motor development

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Motor at T0	21.96 (27.11)	29.11 (20.73)	81.00
Motor at T1	18.20 (20.75)	43.20 (30.18)	51.50*
Motor at T2	22.16 (23.30)	49.00 (23.09)	40.00**
Motor at T3	28.57 (29.19)	56.40 (37.55)	56.00*

**p< .01; *p< .05

2.3. The role of the prenatal experiences in the child's mental development from the time of admission at the institution (T0) onwards (T1, T2, and T3)

Association between Prenatal risk, Birth Weight and Mental development at T0

Spearman correlations revealed no significant associations between prenatal risk and cognitive, language and motor development at T0. Also, birth weight, used as a proxy measure of the risk experienced during prenatal life (that was not included in the composite measure Prenatal Risk) was not significantly associated with cognitive, language and motor performance at T0 (Table 38).

Table 38

Correlations between prenatal risk and birth weight and child's mental development at T0

	Cognitive	Language	Motor
Prenatal Risk (n = 29)	-.10	-.03	-.27
Birth Weight (n = 23)	.15	-.002	.17

Note. Spearman correlations; *p<.05

Association between Prenatal risk, Birth Weight and Mental development at T1

At T1, Prenatal Risk (composite measure) was not significantly associated with any of the domains of mental development (Table 39). On the other hand, birth weight appeared significantly correlated with cognitive performance (r_s= .58, p = .004). This means that in our sample, children were more likely to perform better in cognitive tasks when they were born with higher weight.

Table 39

Correlations between prenatal risk and birth weight and child's mental development at T1

	Cognitive	Language	Motor
Prenatal Risk (n = 29)	-.02	-.03	-.16
Birth Weight (n = 23)	.58**	.32	.35

Note. Spearman correlations; **p<.01; *p<.05.

Association between Prenatal risk, Birth Weight and Mental development at T2

Approximately 5 months after the admission at the institution (T2), Prenatal Risk was not significantly associated with variables of mental development (Table 40). In turn, birth weight was significantly associated with cognitive (r_s= .64, p = .001) and motor (r_s= .49, p = .016) performance, and language functioning (r_s= .37, p = .080), albeit marginally.

Table 40

Correlations between prenatal risk and birth weight and child's mental development at T2

	Cognitive	Language	Motor
Prenatal Risk (n = 29)	-.22	-.12	-.33
Birth Weight (n = 23)	.64**	.37†	.49*

Note. Spearman correlations; **p<.01; *p<.05; †p<.10.

Association between Prenatal risk, Birth Weight and Mental development at T3

At T3, for the first time, prenatal risk appears negatively and significantly associated with cognitive development (r_s= -.55, p = .002), and birth weight was again significantly associated with motor performance (r_s= .56, p = .006), and marginally associated with language functioning (r_s= .37, p = .087), but not with cognitive functioning (Table 41).

Table 41

Correlations between prenatal risk and birth weight and child's mental development at T3

	Cognitive	Language	Motor
Prenatal Risk (n = 29)	-.55**	-.07	-.32
Birth Weight (n = 23)	.28	.37†	.56**

Note. Spearman correlations; *p<.05; †p<.10.

2.4. The role of the prenatal experiences in the child's gain/loss in mental development between T0 and T3

Spearman correlations revealed that higher cognitive differences between T0 and T3, meaning higher gains, tended to be shown by children who had experienced less Prenatal Risk (r_s= -.34, p = .074). In turn, higher gains in motor performance between

T0 and T3 tended to occur for children who were born with more weight (r_s= .44, p = .037) (Table 42) .

Table 42

Correlations between Prenatal risk, Birth Weight and Cognitive, Language and Mental differences between T0 and T3

	Cognitive differences between T0 and T3 (n = 29)	Language differences between T0 and T3 (n = 29)	Motor differences between T0 and T3 (n = 29)
Prenatal Risk (n = 29)	-.34†	-.06	-.21
Birth Weight (n = 23)	.09	.21	.44*

Note. Spearman correlations; *p<.05; †p<.10.

2.5. The role of the early family context risks in the child's mental development from the time of admission at the institution (T0) onwards (T1, T2, and T3)

The early family environment of care was assessed and two composite variables were created as a proxy measure of it. Thus, association tests between family-relational and emotional-negligence risk, were conducted in order to understand their contribution to the child's mental development across time spent in the institution.

Association between Family-relational risk, Emotional-negligence risk and Mental development at T0 (admission at the institution)

Results presented in Table 43 revealed marginally significant associations between family-relational risk experienced before institutionalization and cognitive (r_s= -.35, p = .065) and language (r_s= -.32, p = .087) development at the admission at the institution, meaning that children who experienced more risk in early family context tend to have lower cognitive performance at the institutional placement, while also tended to show higher competences in language. No significant associations were noted between emotional-negligence risk and mental development at T0.

Table 43

Correlations between Family-relational risk, Emotional-negligence risk and child's mental development at T0

	Cognitive	Language	Motor
Family-Relational Risk (n = 29)	-.35†	.32†	-.07
Emotional-Negligence Risk (n = 31)	.03	-.19	-.20

Note. Spearman correlations; †p<.10.

Association between Family-relational risk, Emotional-negligence risk and Mental development at T1

Spearman correlations revealed no associations between family-relational risk, emotional-negligence risk and mental development at T1 (Table 44).

Table 44

Correlations between Family-relational risk, Emotional-negligence risk and child's mental development at T1

	Cognitive	Language	Motor
Family-Relational Risk (n = 29)	.04	.19	.07
Emotional-Negligence Risk (n = 31)	.04	-.11	-.21

Note. Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and Mental development at T2

No associations were found between family-relational risk, emotional-negligence risk and mental development at T2 (Table 45).

Table 45

Correlations between Family-relational risk, Emotional-negligence risk and child's mental development at T2

	Cognitive (n = 29)	Language (n = 29)	Motor (n = 29)
Family-Relational Risk (n = 29)	.18	.19	.02
Emotional-Negligence Risk (n = 31)	.10	-.15	-.09

Note. Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and Mental development at T3

At T3 a single association was found between family-relational risk and language development (r_s= .39, p = .039), suggesting that children who experienced more family-relational risk tended to show better language performance after approximately 8 months of institutionalization (Table 46).

Table 46

Correlations between Family-relational risk, Emotional-negligence risk and child's mental development at T3

	Cognitive	Language	Motor
Family-Relational Risk (n = 29)	.11	.39*	-.02
Emotional-Negligence Risk (n = 31)	-.24	.08	.01

Note. Spearman correlations; *p<.05

2.6. The role of the early family context in the child's gain/loss in mental development between T0 and T3

Also, the role of the early family context was explored in relation to the gains/losses in mental development between T0 and T3.

Results revealed a single significant and positive association between emotional negligence risk and language differences between T0 and T3 (r_s= .44, p = .013). Children who had experienced more emotional-negligence risk were more likely to show higher gains in language performance after the admission at the institution (Table 47).

Table 47

Correlations between Family-relational risk, Emotional-negligence risk and cognitive, language and motor differences between T0 and T3

	Cognitive differences between T0 and T3	Language differences between T0 and T3	Motor differences between T0 and T3
Family-Relational Risk (n = 29)	.30	.18	-.09
Emotional-Negligence Risk (n = 31)	-.18	.44*	.10

2.7. The role of the child's status at the admission at the institution in the child's mental development during institutionalization

Association between Cognitive status at admission and Cognitive functioning at T1, T2 and T3

In the following table (Table 48) are reported associations between the child's status at the admission in terms of cognitive competence at admission, and his/her cognitive functioning at T1, T2, and T3.

Table 48

Correlations between Cognitive status at admission and Cognitive functioning at T1, T2 and T3

	Cognitive T0	Cognitive T1	Cognitive T2	Cognitive T3
Cognitive at T0 (admission) (n = 31)	-	.37*	.24	.14

Note. Spearman correlations; ***p< .001; $^{\dagger}p$< .10.

A single association was found between cognitive status at admission and cognitive functioning at T1 (r_s = .37, p = .042), meaning that higher cognitive performance at the admission at the institution was associated with higher cognitive functioning at T1, but not at T2 and T3.

Association between Language status at admission and Language functioning at T1, T2 and T3

Table 49

Correlations between Language status at admission and Language functioning at T1, T2 and T3

	Language T0	Language T1	Language T2	Language T3
Language at T0 (admission) (n = 31)	-	.62**	.49**	.59**

Note. Spearman correlations; ***p< .001; $^{\dagger}p$< .10.

Language performance at the admission at the institution was associated with language functioning at T1 (r_s = .62, p = .000), at T2 (r_s = .49, p = .005), and at T3 (r_s = .59, p = .001) (Table 49). Results suggest that children with better language development when admitted at the institution were more likely to show better language development while institutionalized.

Association between Motor status at admission and Motor functioning at T1, T2 and T3

Table 50

Correlations between Motor status at admission and Motor functioning at T1, T2 and T3

	Motor T0	Motor T1	Motor T2	Motor T3
Motor at T0 (admission) (n = 31)	-	.53**	.57**	.47**

Note. Spearman correlations; ***p< .001; $^{\dagger}p$< .10.

Finally, significant and positive associations were found between motor performance at the admission and motor performance at T1 (r_s = .53, p = .002), at T2 (r_s = .57, p = .001), and at T3 (r_s = .47, p = .008) (Table 50). This suggests that children at higher levels of motor development at institutional placement (T0) were more likely to show higher levels while institutionalized.

2.8. The role of the child's status at the admission at the institution in the child's gain/loss in mental development between T0 and T3

Association between the child's mental status at the admission and mental gains/losses between T0 and T3 were also analyzed (Table 51).

Table 51

Correlations between Cognitive, Language, Motor functioning at admission and cognitive, language and motor differences between T0 and T3

	Cognitive differences between T0 and T3	Language differences between T0 and T3	Motor differences between T0 and T3
Cognitive percentile at T0 (admission) (n = 31)	-.66**	-	-
Language percentile at T0 (admission) (n = 31)	-	-.24	-
Motor percentile at T0 (admission) (n = 31)	-	-	-.10

Note. Spearman correlations; **p< .01

Cognitive competence at the admission was negatively and significantly associated with differences between T0 and T3 in cognitive performance (r_s= .66, p = .000), meaning that children with lower percentiles in cognitive assessment at T0 tended to show higher gains between T0 and T3 in their cognitive competence (Table 51). The ones that entered at the worse condition are the ones who benefit more from being institutionalized, in terms of cognitive gains. No associations were found between

language development at T0 and language gains/losses between T0 and T3, and the same was true for motor development.

2.9. The role of the quality of institutional context in the child's mental development during institutionalization (T1, T2, and T3)

As it was the case for physical growth, the variables regarding the assessment of the quality of the institutional context were only associated with mental development at T1, T2 and T3, since at T0 the child's mental functioning was, in our view, more related to his/her life history in early family context than to the quality of care provided in the institution.

Association between institutional context variables and cognitive, language and motor performance at T1

In Table 52 are presented the associations between child's mental development at T1, in terms of child's cognitive, language and motor performance and the variables of the quality of institutional and caregiving context where the child was integrated. A single significant association was found between the level of developmental activities promoted by the institution and motor development (r_s = .38, p = .038). Institutions where there were more developmental activities children tended to show higher motor development at T1.

Table 52

Correlations between mental development at T1 and institutional quality context

	Cognitive T1	**Language T1**	**Motor T1**
Human Resources	.03	.24	.02
Equipment and material resources	.17	.10	.24
Basic Needs Routines	-.08	.06	.01
Developmental activities	.16	.19	.38*
Caregiving stability	.21	.02	-.01

Note. Spearman correlations; *p< .05

Association between institutional context variables and cognitive, language and motor performance at T2

The table that follows (Table 53) presents the Spearman associations between the quality of institutional context and mental development at T2. A significant and positive association was found between the quantity of human resources working at the institution and the child's language performance at T2 (r_s = .38, p = .037), meaning that institutions with higher number of human resources were more likely to have children with better language development. Also, more developmental activities promoted by the institution were associated with child's higher motor capacities at T2 (r_s = .37, p = .039).

Table 53

Correlations between mental development at T2 and institutional quality context

	Cognitive T2	**Language T2**	**Motor T2**
Human Resources	-.18	.38*	.07
Equipment and material resources	.18	.35	.27
Basic Needs Routines	-.10	.22	-.04
Developmental activities	.30	.26	.37*
Caregiving stability	.07	-.14	.15

Note. Spearman correlations; *p< .05

Association between institutional context variables and cognitive, language and motor performance at T3

At T3 a single association was found between motor development and the quantity and quality of the developmental activities promoted (r_s = .38, p = .034) (Table 54). Thus, institutions where developmental activities were more promoted tended to have children with better motor development at T3.

Table 54

Correlations between mental development at T3 and institutional quality context

	Cognitive T3	**Language T3**	**Motor T3**
Human Resources	-.10	-.13	.03
Equipment and material resources	.27	.30	.33
Basic Needs Routines	.12	.01	-.02
Developmental activities	.17	.15	.38*
Caregiving stability	-.32	-.04	.06

Note. Spearman correlations; *p< .05

2.10. The role of the quality of institutional context in the child's gain/loss in mental development between T0 and T3

Associations between the quality of institutional context and mental gains/losses between T0 and T3 were next presented (Table 55).

Results revealed a significant association between institutional human resources and the magnitude of gain/loss in language development between T0 and T3 (r_s= -.36, p = .049), suggesting that at institutions with less human resources the children's gains in language development were higher than in institutions where there were more human resources. Another significant association was found, between caregiving stability and cognitive gains/losses between T0 and T3 (r_s= -.40, p = .027), meaning that institutions where there were lower stability and consistency in the caregiving children tended to reveal higher gains in cognitive performance between T0 and T3.

Table 55

Correlations between institutional quality context and cognitive, language and motor differences between T0 and T3

	Cognitive differences between T0 and T3	Language differences between T0 and T3	Motor differences between T0 and T3
Human Resources	-.14	-.36*	-.05
Equipment and material resources	-.07	.19	.15
Basic Needs Routines	.19	.17	.03
Developmental activities	-.04	.19	.22
Caregiving stability	-.40*	-.12	-.14

Note. Spearman correlations; *p< .05

2.11. The role of the caregiver's sensitivity and cooperation behaviors in the child's mental development during institutionalization (T1, T2, and T3)

Association between caregiver's sensitivity and cooperation in play interaction with the child and cognitive, language and motor performance at T1

Similarly to what happened above, also for caregiver's sensitivity and cooperation analyses were only conducted from T1 onwards.

Spearman correlations revealed no significant associations between caregiver's sensitivity in interaction with the child and his/her mental development at T1 (Table 56). However, caregiver's cooperation revealed to be marginally associated with language development (r_s= -.36, p = .062). This result suggests that children who experienced higher cooperative caregiving behaviors tended to show lower language competence at T1.

Table 56

Correlations between caregiver's sensitivity and cooperation and mental development at T1

	Cognitive T1	**Language T1**	**Motor T1**
Sensitivity (n = 28)	-.23	-.16	.04
Cooperation (n = 28)	-.31	-.36†	-.18

Note. Spearman correlations; $^{\dagger}p < .10$

Association between caregiver's sensitivity and cooperation in play interaction with the child and cognitive, language and motor performance at T2

In what concerns the associations between caregiver's sensitivity and cooperation and child's mental development at T2, Spearman correlations (Table 57)

revealed significant negative associations between caregiver's sensitivity and cognitive functioning (r_s= -.34, p = .064), and also between caregiver's cooperation and child's cognitive functioning (r_s= -.31, p = .095), suggesting that children cared by more sensitive and cooperated caregivers were more likely to show worse cognitive performance ate T2

Table 57

Correlations between caregiver's sensitivity and cooperation and mental development at T2

	Cognitive T2	**Language T2**	**Motor T2**
Sensitivity (n = 31)	-.34†	-.05	-.16
Cooperation (n = 30)	-.31†	-.21	-.06

Note. Spearman correlations; †p< .10

Association between caregiver's sensitivity and cooperation in play interaction with the child and cognitive, language and motor performance at T3

At T3 no significant associations were verified between the caregiver's sensitivity and cooperation behaviors and child's mental development (Table 58).

Table 58

Correlations between caregiver's sensitivity and cooperation and mental development at T3

	Cognitive T3	**Language T3**	**Motor T3**
Sensitivity (n = 30)	.21	.001	.11
Cooperation (n = 30)	.13	-.07	-.02

Note. Spearman correlations

2.12. The role of the caregiver's sensitivity and cooperation behaviors in the child's gain/loss in mental development between T0 and T3

Correlations between the magnitude of gains/losses in mental development between T0 and T3 and caregiver's sensitivity and cooperation behaviors were performed. The measurement of caregiver's sensitivity and cooperation at T0 was included, and excluded the measurement at T3, for the same reasons described above.

Spearman correlations revealed a single significant and negative association between caregiver's cooperative behaviors at T0 and the magnitude of gains/losses in motor development between T0 and T3 (r_s= -.45, p = .023), suggesting that children who had experienced lower levels of caregiver's cooperation at T0 tended to show higher gains in motor development during the period of institutionalization (Table 59).

Table 59

Correlations between institutional quality context and height, weight and head circumference differences between T0 and T3

	Cognitive differences between T0 and T3	Language differences between T0 and T3	Motor differences between T0 and T3
Sensitivity T0 (n=25)	-.04	-.02	-.30
Cooperation T0 (n=25)	-.20	-.27	-.45*
Sensitivity T1 (n=28)	.32	.05	-.03
Cooperation T1 (n=28)	.19	.23	-.05
Sensitivity T2 (n=31)	-.19	-.05	-.29
Cooperation T2 (n=30)	-.17	.05	-.19
Sensitivity T3 (n=30)	.14	.27	.08
Cooperation T3 (n=30)	-.02	.21	-.04

Note. Spearman correlations; *p< .05

3. NEUROENDOCRINE FUNCTIONING: DIURNAL CORTISOL PRODUCTION AND REGULATION

3.1. Evolution across time for diurnal cortisol production and regulation

The evolution of cortisol production over the time spent in the institution was analyzed as follows.

3.1.1. Evolution of cortisol levels and variation across the day (morning, noon and afternoon) in each moment of assessment (T0, T1, T2, and T3)

The diurnal pattern of cortisol production, in terms of their variation across the day in each moment of assessment was analyzed as follows (Figure 22).

Cortisol levels and variation across the day at Time 0

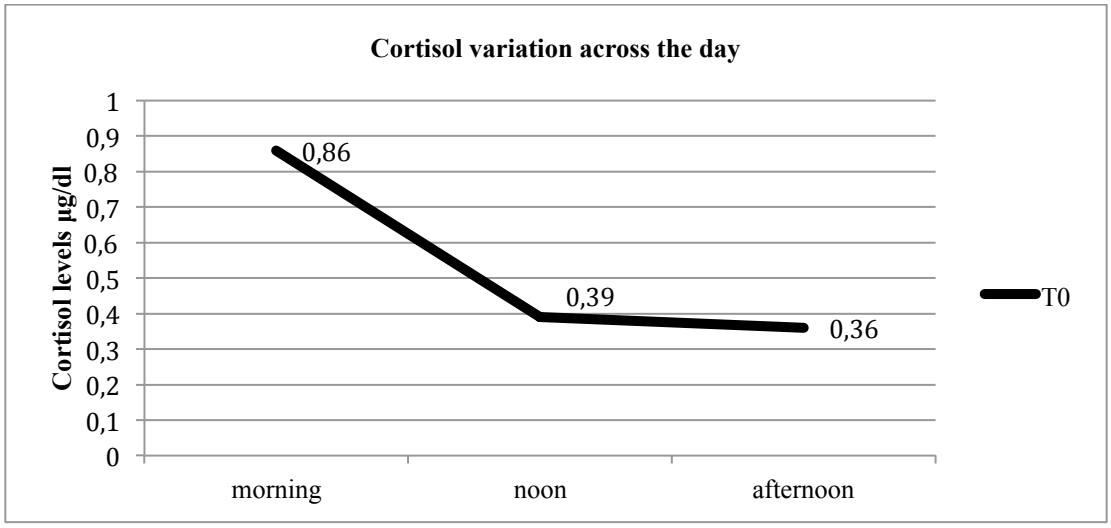

Figure 22: Cortisol variation across the day at T0

At the admission at the institution (T0) there was significant variation in cortisol levels across the day, χ^2 (2) = 16.21, p = .000.

Cortisol levels and variation across the day at Time 1

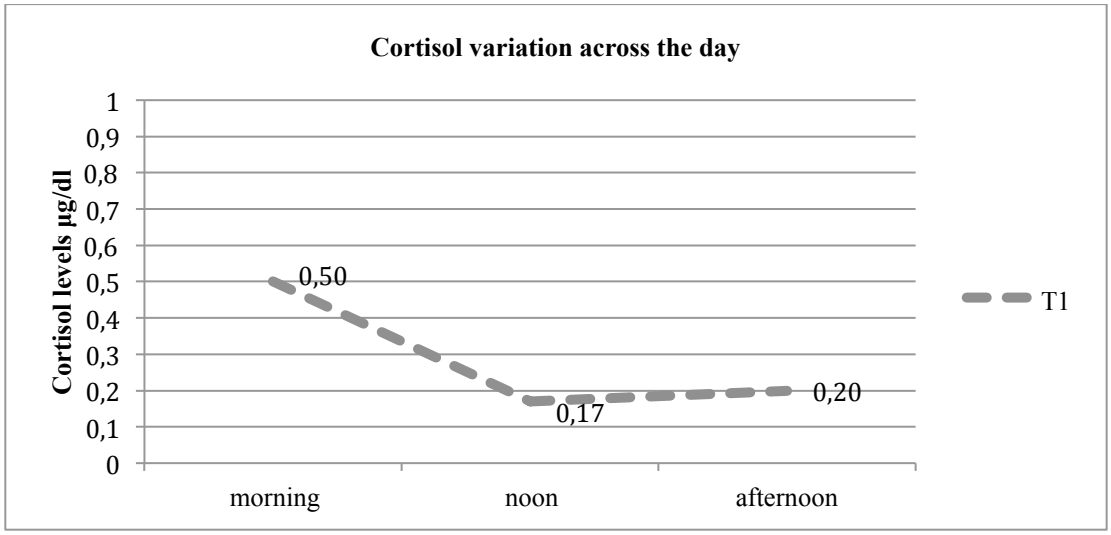

Figure 23: Cortisol variation across the day at T1

At T1 there was significant variation in cortisol levels across the day, χ^2 (2) = 23.87, p = .000 (Figure 23).

Cortisol levels and variation across the day at Time 2

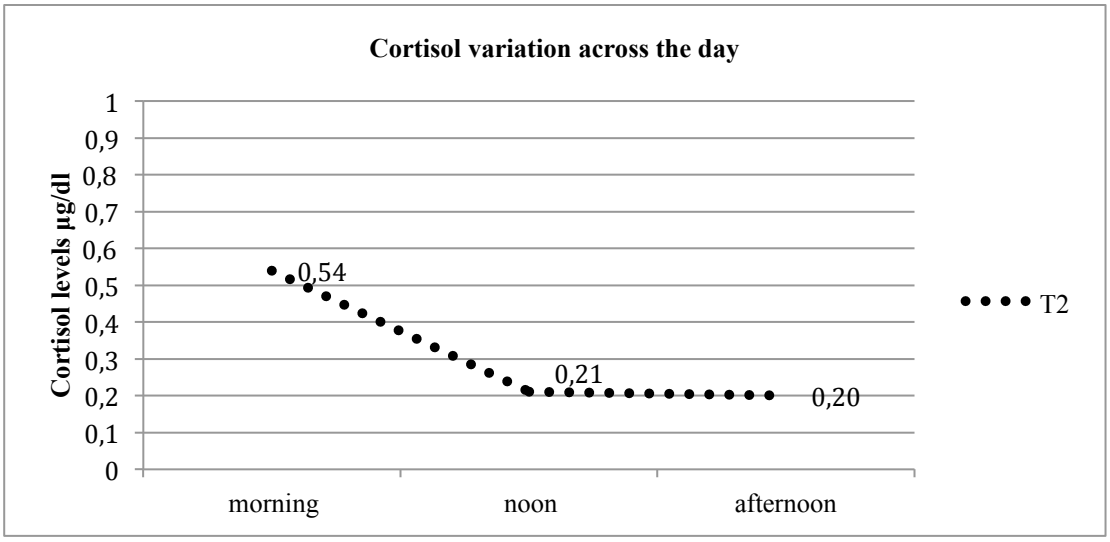

Figure 24: Cortisol variation across the day at T2

At T2 there was significant variation in cortisol levels across the day, χ^2 (2) = 20.02, p = .000 (Figure 24).

Cortisol levels and variation across the day at Time 3

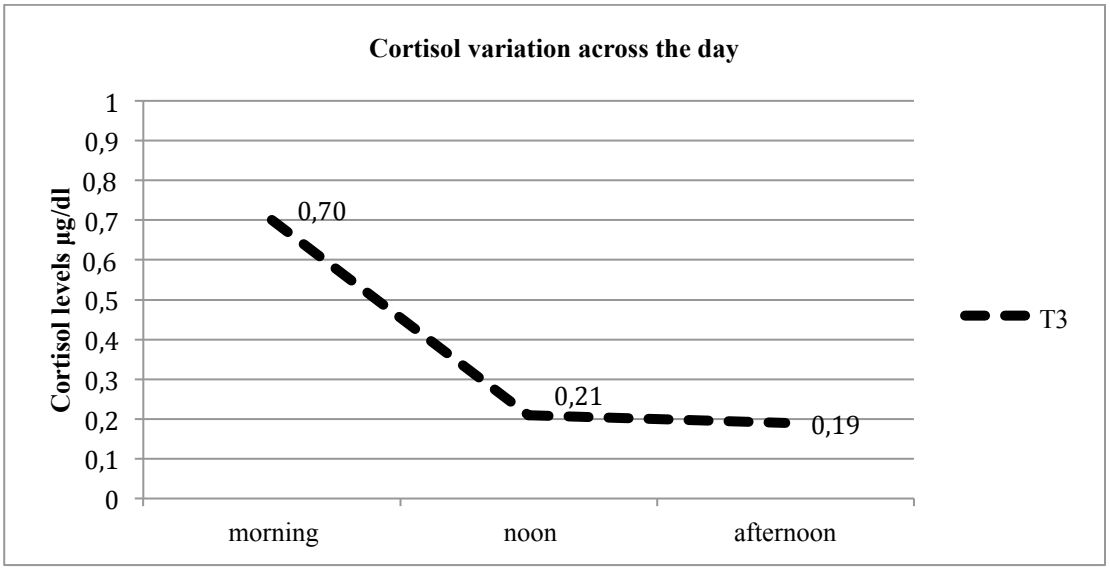

Figure 25: Cortisol variation across the day at T3

Finally, at T3 there was significant variation in cortisol levels across the day, χ^2 (2) = 32.97, p = .000 (Figure 25).

Differences between moments of assessment (T0, T1, T2, and T3) in each time point of the day (morning, noon and afternoon)

The Figure 26 presents the overlapping between each moment of assessment of the daily cortisol levels and variation..

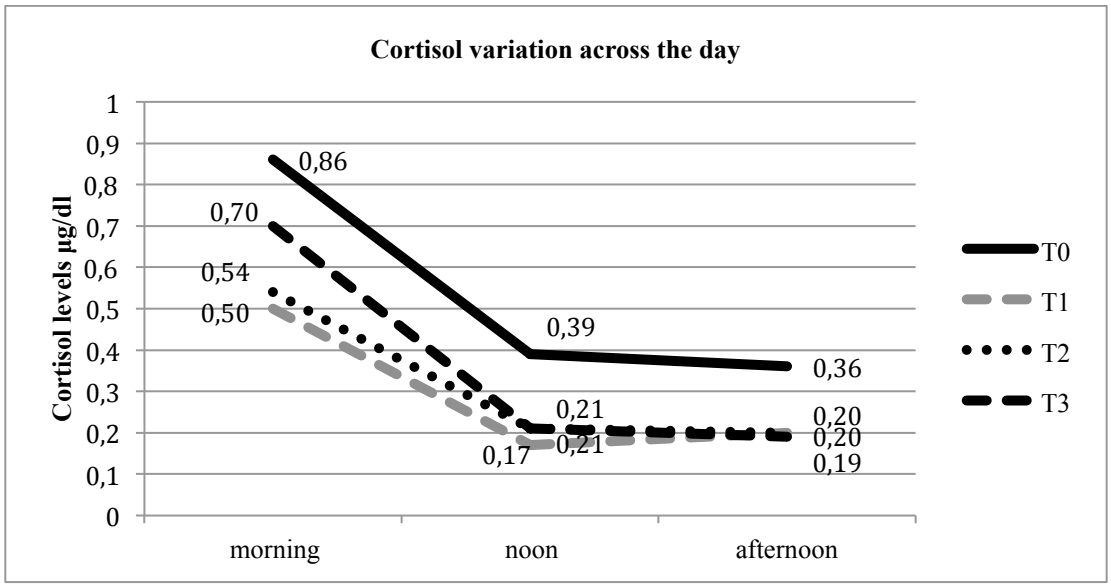

Figure 26: Cortisol variation across the day at T0, T1, T2 and T3

The following table (Table 60) presents results from the differences across time spent in institution for morning, noon and afternoon cortisol levels.

Table 60

Differences across time between morning, noon and afternoon cortisol levels

	Moments of assessment				
	T0 (n = 31) *Mean (SD)*	T1 (n = 31) *Mean (SD)*	T2 (n = 31) *Mean (SD)*	T3 (n = 31) *Mean (SD)*	$\chi^2(3)$
Morning Cortisol	.86 (.63)	.50 (.34)	.54 (.37)	.70 (.42)	8.36*
Noon Cortisol	.39 (.53)	.17 (.15)	.21 (.20)	.21 (.18)	6.03
Afternoon Cortisol	.36 (.40)	.20 (.25)	.20 (.23)	.19 (.16)	9.09*

* $p < .05$

There were differences across time spent in the institution (T0, T1, T2 and T3) only for morning cortisol levels, χ^2 (3) = 8.36, p = .04, and afternoon cortisol levels, χ^2 (3) = 9.09, p = .03 (Table 60).

To determine where the differences occurred, additional analyses were conducted. The results were as follows in table 61. For morning cortisol levels the main difference was between T0 and T1, when the levels were the highest and the lowest respectively, Z = -2.54, p = .011, and at T0 and T2, Z = -1.99, p = .05. Between T1 and T3 there was a significant increase in morning cortisol levels, Z = -2.04, p = .04. There was no significant differences in morning cortisol levels between T0 and T3 levels, which suggests an approaching to baseline levels after around 8 months of institutionalization, Z = -.93, p = .35. For noon cortisol levels the main differences were between T0 and T1, Z = -2.31, p = .02, and between T0 and T2, Z = -2.01, p = .05. Again there was an approach to the baseline levels at T3, Z = -1.36, p = .17. In turn, for afternoon cortisol levels the main differences were between T0 and T1, Z = -2.50, p = .01, and between T0 and T2, Z = -2.89, p = .004. Differences between T0 and T3 were only marginally significant, Z = -1.78, p = .07, which also suggests an approach to the highest levels registered at T0. The overall picture seems to suggest that cortisol levels at T3 were similar to the ones showed at the admission at the institution (T0).

Table 61

Comparisons between moments of assessment for morning, noon and afternoon cortisol levels

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
Morning Cortisol	*	*	*ns*	*ns*	*	*ns*
Noon Cortisol	*	*	*ns*	*ns*	*ns*	*ns*
Afternoon Cortisol	**	**	†	*ns*	*ns*	*ns*

** $p < .01$; * $p < .05$; † $p < .10$

3.1.2. Evolution of diurnal cortisol production (AUCg) across time spent in the institution

It is next presented (Figure 27) the evolution of diurnal overall cortisol production (AUCg) and the statistical results regarding the differences across time spent in the institution.

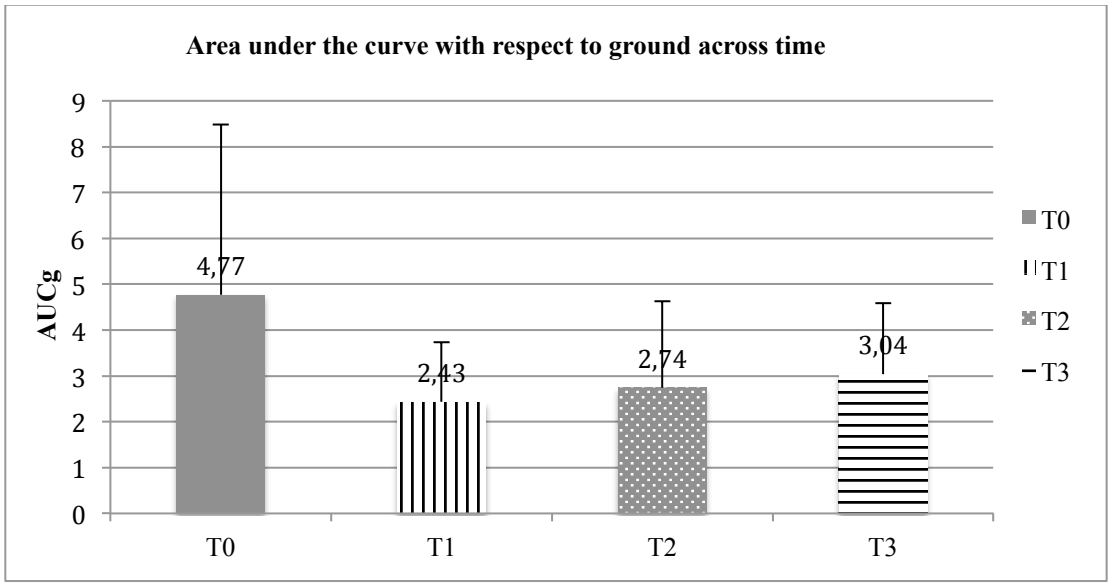

Figure 27: Evolution across time for the AUCg

The mean value at T0 was 4.77 (*SD* = 3.71), at T1 was 2.43 (*SD* = 1.30), at T2 was 2.74 (*SD* = 1.89), and at T3 was 3.04 (*SD* = 1.55). There were significant differences in terms of cortisol production, across time spent in the institution, χ^2 (3) = 20.16, p = .000.

Table 62 shows the comparisons between moments of assessment for AUCg. Again, the AUCg values at T3 suggested an approach to the admission levels (T0), which were the highest across time spent in the institution.

Table 62

Comparisons between moments of assessment for AUCg values

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
AUCg	**	**	†	*ns*	*ns*	*ns*

** $p < .01$; † $p < .10$

Intra-individual changes between T0 and T3 were also analyzed for AUCg levels (Figure 28).

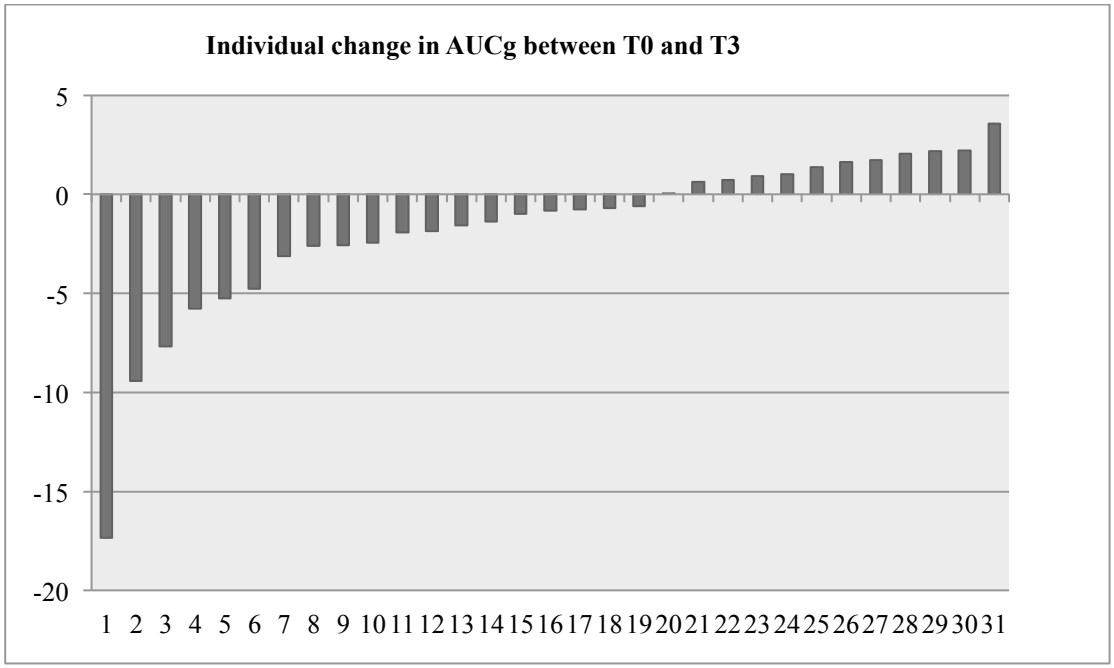

Figure 28: Intra-individual changes in AUCg values across time

3.1.3. Evolution of cortisol regulation across time spent in the institution

In the following table (Table 63) are presented the results from the analysis of the differences across time for cortisol regulation.

Table 63

Differences across time for cortisol regulation

	Moments of assessment				
	T0 (n = 31) *Mean (SD)*	T1 (n = 31) *Mean (SD)*	T2 (n = 31) *Mean (SD)*	T3 (n = 31) *Mean (SD)*	χ^2 (3)
Cortisol Regulation	.51 (.64)	.30 (.36)	.34 (.37)	.50 (.43)	9.99*

* $p < .05$

There were significant differences across time spent in the institution in cortisol regulation, χ^2 (3) = 9.99, p = .019 (Table 63).

These differences were marginally significant between T0 and T1, when a decrease was noted, and between T1 and T3, when an increase occurred (Table 64).

Table 64

Comparisons between moments of assessment for cortisol regulation

	Moments of assessment					
	T0 *vs.* T1	**T0 *vs.* T2**	**T0 *vs.* T3**	**T1 *vs.* T2**	**T1 *vs.* T3**	**T2 *vs.* T3**
Cortisol Regulation	†	*ns*	*ns*	*ns*	†	*ns*

*** $p < .001$; ** $p < .01$

Intra-individual changes were also analyzed and results are depicted as follow (Figure 29).

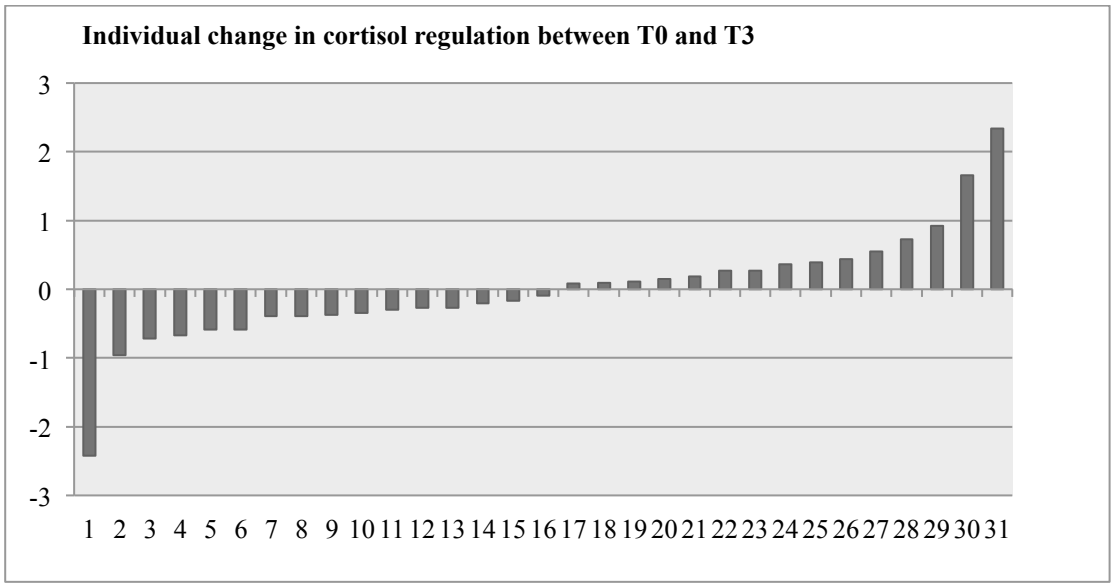

Figure 29: Intra-individual changes in cortisol regulation across time

Negative differences between T0 and T3 indicate that children have lost some of their ability to regulate cortisol production during the day between the two time points. In other words, they have lost some of their capacity to diminish cortisol production between morning and afternoon hours. In our sample, 16 children (51.61%) presented that pattern. Conversely, 15 children (48.39%) showed gains between the admission and T3 assessment in the ability to regulate diurnal cortisol production, meaning that at T3 they were more able to diminish cortisol levels across the day.

3.2. Effect of age at admission in cortisol production (morning, noon, and afternoon levels and AUCg) and cortisol regulation across time spent in the institution

The effect of age at admission was also tested for diurnal cortisol production and regulation, using the dichotomized age with the cutoff point at 12 months. The results are presented in Figure 30 and Table 65.

Area Under the Curve with respect to ground - AUCg

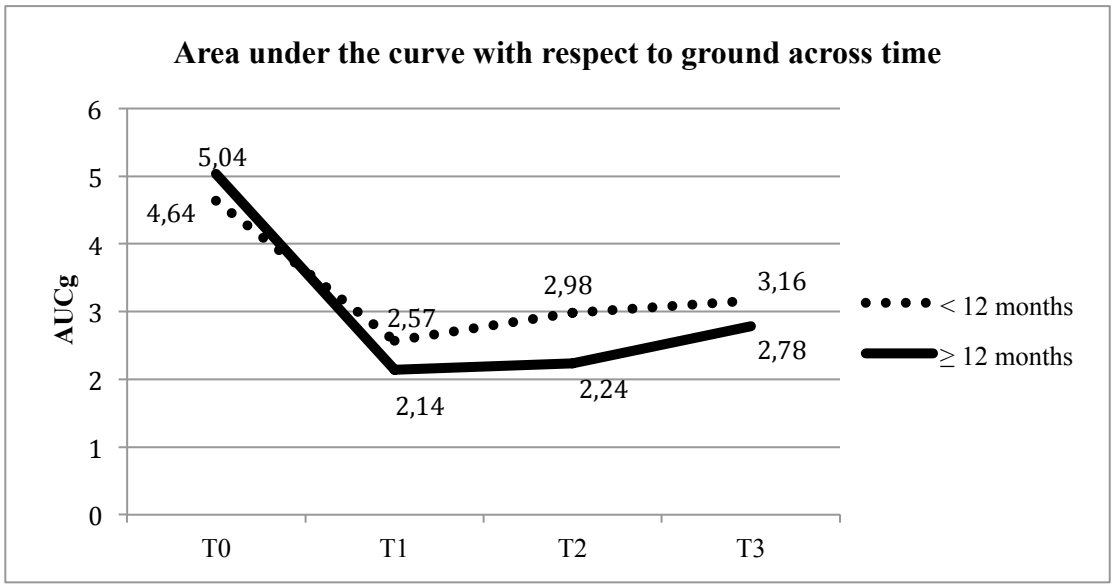

Figure 30: AUCg differences between the two age groups (below or above 12 months) across time

Analysis of the evolution across time revealed significant changes for the children admitted older (above 12 months) χ^2 (3) = 9.03, p = .03. Significant decreases occurred between T0 and T1 (Z = -2.60, p = .009) and between T0 and T2 (z = -2.31, p = .021). Significant changes were also noted for the children admitted earlier (below 12 months) χ^2 (3) = 11.64, p = .009, with significant decreases between T0 and T1 (Z = -2.31, p = .021) and between T0 and T2 (z = -2.40, p = .016).

In terms of the differences between groups in each moment of assessment the analyses of the differences (Table 66), revealed no significant differences between the groups at T0 (U = 98.50, p = .787), at T1 (U = 93.00, p = .633), at T2 (U = 88.50, p = .492), and at T3 (U = 84.50, p = .393).

Table 65

Differences between age groups in AUCg

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
AUCg at T0	4.64 (3.88)	5.04 (3.51)	98.50
AUCg at T1	2.57 (1.45)	2.14 (.90)	93.00
AUCg at T2	2.98 (2.18)	2.24 (.92)	88.50
AUCg at T3	3.16 (1.64)	2.78 (1.39)	84.50

Cortisol levels at 7 am, 11 am, and 5 pm

Analyses of the differences in terms of the cortisol levels at 7 am, 11 am and 5 pm, and evolution across time for each of the age group were conducted and the results are showed next.

Cortisol levels at 7 am

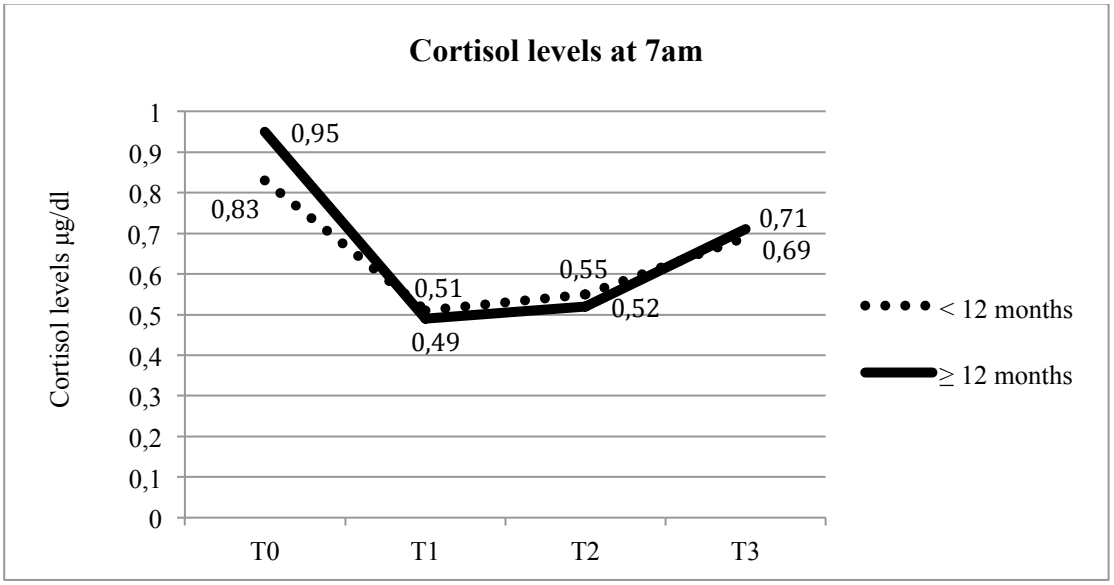

Figure 31: Differences in the morning cortisol between the two age groups (below or above 12 months) across time

Analyses of the evolution across time for each group revealed significant changes for the children admitted older χ^2 (3) = 9.77, p = .02, occurring between T0 and T1 (Z = -2.09, p = .037) and between T0 and T2 (Z = -2.43, p = .015). Contrarily, no significant changes for the group admitted earlier than 12 months were noted χ^2 (3) = 1.91, p = .59.

In terms of the differences between groups in each moment of assessment (Table 66), no significant differences were found at T0 (U = 79.50, p = .287), at T1 (U = 100.50, p = .852), at T2 (U = 98.00, p = .787), and at T3 (U = 85.50, p = .416).

Table 66

Differences between age groups in 7 am cortisol levels

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Cortisol 7am at T0	.83 (.69)	.95 (.51)	79.50
Cortisol 7am at T1	.51 (.34)	.49 (.37)	100.50
Cortisol 7am at T2	.55 (.37)	.52 (.39)	98.00
Cortisol 7am at T3	.69 (.49)	.71 (.25)	85.50

Cortisol levels at 11 am

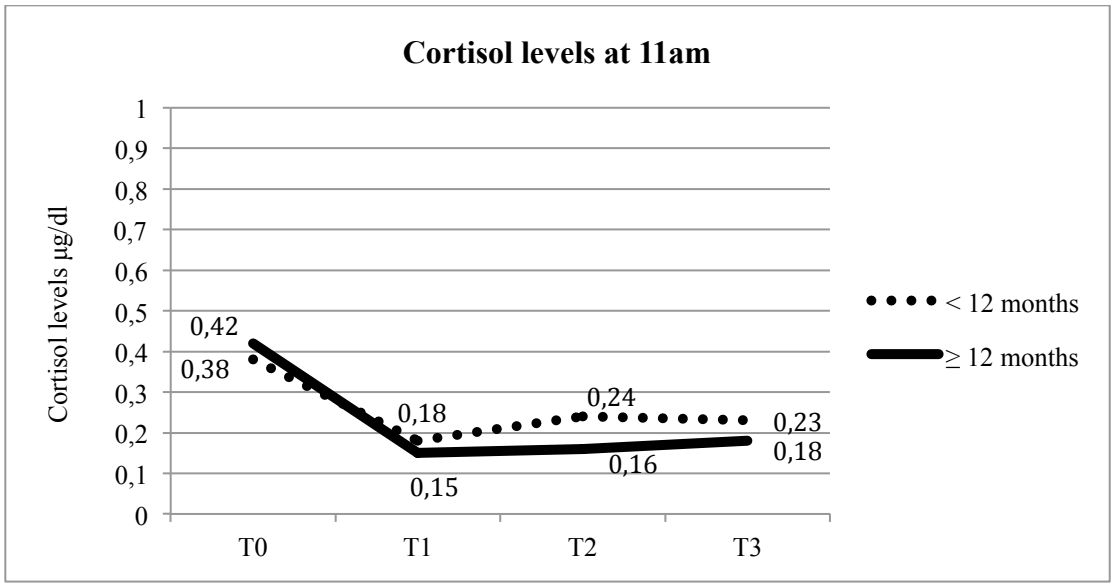

Figure 32: Differences in the noon cortisol between the two age groups (below or above 12 months) across time

No significant changes across time were noted for the children admitted older χ^2 (3) = 1.87, p = .60, although a marginally significant difference was noted between T0 and T1 (Z = -1,69, p = .092). No significant changes across time for the children admitted earlier χ^2 (3) = 4.31, p = .23, although a marginally significant difference was noted between T0 and T1 (Z = -1,70, p = .09) and another between T0 and T3 (Z = -1.70, p = .09).

Regarding the differences between the two age groups in each moment of assessment (Table 67), no significant differences were found at T0 (U = 92.00, p = .603), at T1 (U = 80.00, p = .306), at T2 (U = 89.50, p = .519), and at T3 (U = 78.50, p = .268).

Table 67

Differences between age groups in 11 am cortisol levels

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Cortisol 11am at T0	.38 (.57)	.42 (.46)	92.00
Cortisol 11am at T1	.18 (.18)	.15 (.05)	80.00
Cortisol 11am at T2	.24 (.24)	.16 (.08)	89.50
Cortisol 11am at T3	.23 (.19)	.18 (.15)	78.50

Cortisol levels at 5 pm

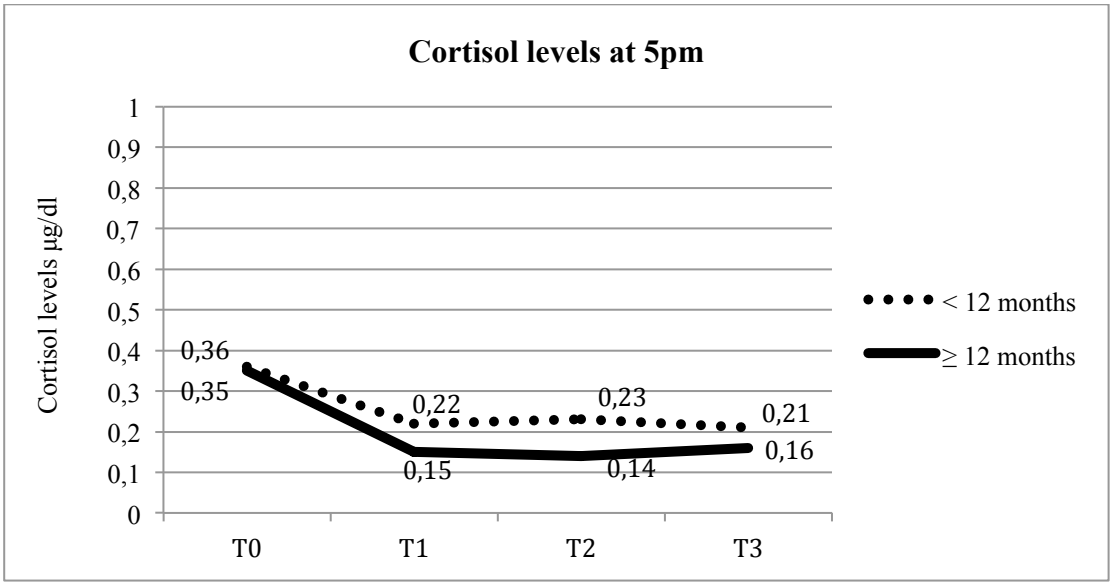

Figure 33: Differences in the afternoon cortisol between the two age groups (below or above 12 months) across time

No significant changes for the group of children admitted older than 12 months were noted χ^2 (3) = 4.30, p = .23, although pairwise comparisons revealed significant differences between T0 and T1 (Z = -1.99, p = .047), between T0 and T2 (Z = -1.96, p = .05), and also between T0 and T3 (Z = -1.78, p = .075). Analyses also revealed no significant changes across time for the children admitted earlier χ^2 (3) = 5.49, p = .14, although a significant difference was noted between T0 and T2 (Z = -2.13, p = .033).

Regarding the differences between the two age groups (Table 68), analyses also revealed no significant differences for the 5 pm cortisol levels at T0 (U = 98.50, p =

.787), at T1 (U = 97.00, p = .755), at T2 (U = 94.50, p = .663), and at T3 (U = 79.00, p = .287).

Table 68

Differences between age groups in 5 pm cortisol levels

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Cortisol 5pm at T0	.36 (.42)	.35 (.36)	98.50
Cortisol 5pm at T1	.22 (.30)	.15 (.08)	97.00
Cortisol 5pm at T2	.23 (.27)	.14 (.08)	94.50
Cortisol 5pm at T3	.21 (.17)	.16 (.12)	79.00

Cortisol regulation

Analyses of the differences between the two age groups were conducted for the variable cortisol regulation, as well as analyses of the differences across time for each age group. Results are as follows.

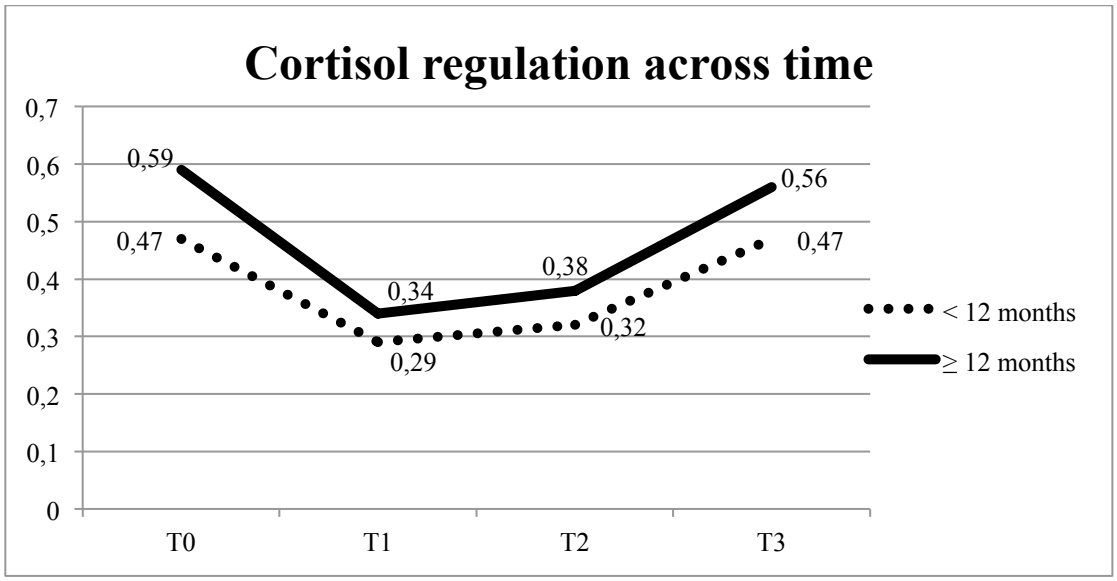

Figure 34: Cortisol regulation differences between the two age groups (below or above 12 months) across time

Significant changes were noted for the children admitted older than 12 months χ^2 (3) = 7.79, p = .05, with pairwise comparisons revealing only a marginally significant decrease between T0 and T2 (Z = -1.72, p = .086). In turn, no significant changes

occurred across time for the children admitted earlier than 12 months χ^2 (3) = 3.96, p = .266.

Analyses of the differences between the two age groups (Table 69) revealed no significant differences in in terms of the cortisol regulation, at T0 (U = 91.50, p = .574), at T1 (U = 103.50, p = .950), at T2 (U = 95.50, p = .693), and at T3 (U = 80.50, p = .306).

Table 69

Differences between age groups in cortisol regulation

	Age at admission		
	< 12 months (n = 21) *Mean (SD)*	≥ 12 months (n = 10) *Mean (SD)*	U
Cortisol regulation at T0	.47 (.75)	.59 (.36)	91.50
Cortisol regulation at T1	.29 (.39)	.34 (.33)	103.50
Cortisol regulation at T2	.32 (.36)	.38 (.40)	95.50
Cortisol regulation at T3	.47 (.51)	.56 (.21)	80.50

3.3. The role of the prenatal experiences in the child's diurnal cortisol production (morning, noon, and afternoon levels and AUCg) and cortisol regulation across time spent in the institution

Association between Prenatal risk, Birth Weight and Cortisol production and regulation at T0

Next table (Table 70) presents the associations between prenatal risk and birth weight and cortisol variables. Spearman correlations revealed positive and significant associations between prenatal risk and morning cortisol levels (r_s= .45, p = .014), between prenatal risk and noon cortisol (r_s= -.41, p = .028), and between prenatal risk and the magnitude of cortisol decrease across the day (r_s= -.49, p = .007). Results suggest that children exposed to higher prenatal risk tended to show higher morning cortisol and lower noon cortisol levels. Besides that it seems that the higher the prenatal risk experienced the higher the decrease across the day at the admission at the institution.

Significant associations between birth weight and noon cortisol (r_s= .53, p = .010), and birth weight and overall diurnal production (AUCg) (r_s= .48, p = .022) were also noted, meaning that children who born with higher weight tended to show higher noon cortisol and higher overall diurnal production when admitted at the institution.

Table 70

Correlations between prenatal risk and birth weight and cortisol production and regulation at T0

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Prenatal Risk (n = 29)	.45*	-.41*	-.08	-.08	.49**
Birth Weight (n = 23)	-.11	.53**	.24	.48*	-.11

Note. Spearman correlations; **p < .01; * p < .05;

Association between Prenatal risk, Birth Weight and Cortisol production and regulation at T1

Table 71 presents the associations between prenatal risk and birth weight and cortisol variables at T1. Results revealed no significant associations between the variables tested.

Table 71

Correlations between prenatal risk and birth weight and cortisol production and regulation at T1

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Prenatal Risk (n = 29)	.09	.14	-.07	.18	.06
Birth Weight (n = 23)	-.05	.04	-.24	-.28	.16

Note. Spearman correlations

Association between Prenatal risk, Birth Weight and Cortisol production and regulation at T2

Statistical analysis revealed no significant associations between the variables tested (Table 72).

Table 72

Correlations between prenatal risk and birth weight and cortisol production and regulation at T2

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Prenatal Risk (n = 29)	-.22	-.10	.01	-.18	-.17
Birth Weight (n = 23)	.29	.08	.31	.30	.19

Note. Spearman correlations ; $^{\dagger}p<.10$.

Association between Prenatal risk, Birth Weight and Cortisol production and regulation at T3

Spearman correlations (Table 73) revealed no significant associations between the variables tested.

Table 73

Correlations between prenatal risk and birth weight and cortisol production and regulation at T3

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Prenatal Risk (n = 29)	.13	-.03	.29	.19	.04
Birth Weight (n = 23)	-.03	-.01	.04	.03	-.02

Note. Spearman correlations

3.4. The role of the prenatal experiences in the differences in cortisol production between T0 and T3

A single marginally significant association was found between prenatal risk and differences between T0 and T3 in child's ability to regulate cortisol across the day (r_s= -.33, p = .083), meaning that children exposed to higher prenatal risk tended to diminish their capacity to regulate cortisol between T0 and T3 (Table 74).

Table 74

Correlations between prenatal risk and cortisol production and regulation differences between T0 and T3

	AUCg differences between T0 and T3 (n = 29)	Cortisol regulation differences between T0 and T3 (n = 29)
Prenatal Risk (n = 29)	.24	-.33†
Birth Weight (n = 23)	-.32	-.01

Note. Spearman correlations; $^{\dagger}p$<.10.

3.5. The role of the early family context risks in the child's diurnal cortisol production (morning, noon, and afternoon levels and AUCg) and cortisol regulation across time spent in the institution

Association between Family-relational risk, Emotional-negligence risk and cortisol production and regulation at T0 (admission at the institution)

No significant associations were found between family-relational risk, emotional-negligence risk and cortisol variables measured at the admission at the institution (Table 75).

Table 75

Correlations between Family-relational risk, Emotional-negligence risk and child's cortisol production and regulation at T0

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Family-Relational Risk (n = 29)	-.22	-.22	-.12	-.19	-.19
Emotional-Negligence Risk (n = 31)	.29	-.13	.23	.24	.18

Note: Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and cortisol production and regulation at T1

Also, at T1, there were no significant associations between family-relational risk and emotional-negligence risk and the cortisol levels and regulation (Table 76).

Table 76

Correlations between Family-relational risk, Emotional-negligence risk and child's cortisol production and regulation at T1

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Family-Relational Risk (n = 29)	.10	.07	.21	.26	.07
Emotional-Negligence Risk (n = 31)	.08	-.10	-.14	-.19	.20

Note: Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and cortisol production and regulation at T2

Associations between early family context experiences and cortisol variables at T2 were not significant (Table 77).

Table 77

Correlations between Family-relational risk, Emotional-negligence risk and child's cortisol production and regulation at T2

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Family-Relational Risk (n = 29)	-.07	-.12	-.08	-.09	-.13
Emotional-Negligence Risk (n = 31)	-.02	.02	-.04	-.12	.03

Note: Spearman correlations

Association between Family-relational risk, Emotional-negligence risk and cortisol production and regulation at T3

Table 78 present no significant associations between both family-relational and emotional-negligence risks experienced before institutionalization and cortisol levels and regulation around 8 months after being institutionalized (T3).

Table 78

Correlations between Family-relational risk, Emotional-negligence risk and child's cortisol production and regulation at T3

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Family-Relational Risk (n = 29)	.11	-.19	.14	.14	.08
Emotional-Negligence Risk (n = 31)	.28	-.19	.11	.15	.19

Note: Spearman correlations

3.6. The role of the early family context risks in the differences in cortisol production and regulation between T0 and T3

Spearman correlations revealed no significant association between both family-relational and emotional-negligence risk and the differences in cortisol production (AUCg) and regulation between T0 and T3 (Table 79).

Table 79

Correlations between Family-relational risk, Emotional-negligence risk and cortisol production and regulation differences between T0 and T3

	AUCg differences between T0 and T3	Cortisol regulation differences between T0 and T3
Family-Relational Risk (n = 29)	.21	.17
Emotional-Negligence Risk (n = 31)	.10	.04

Note. Spearman correlations

3.7. The role of the child's status at the admission at the institution in the child's cortisol production (AUCg) and cortisol regulation during institutionalization

Association between Diurnal cortisol production (AUCg) at admission and Diurnal cortisol production (AUCg) at T1, T2 and T3

No significant associations were found between the diurnal cortisol production (AUCg) at admission and the diurnal cortisol production in the following assessments (Table 80).

Table 80

Correlations between AUCg levels at admission and AUCg levels at T1, T2 and T3

	AUCg T0	AUCg T1	AUCg T2	AUCg T3
AUCg at T0 (admission) (n = 31)	-	-.18	.27	.01

Note. Spearman correlations

Association between Cortisol regulation at admission and Cortisol regulation at T0, T1, T2 and T3

No significant associations were noted between the variables tested (Table 81).

Table 81

Correlations between Cortisol regulation at admission and Cortisol regulation at T1, T2 and T3

	Cortisol Regulation T0	Cortisol Regulation T1	Cortisol Regulation T2	Cortisol Regulation T3
Cortisol Regulation at T0 (admission) (n = 31)	-	.16	-.11	.11

Note. Spearman correlations

3.8. The role of the child's status at the admission at the institution in the differences in cortisol production and regulation between T0 ad T3

Results presented in the next table (Table 82) revealed a significant and negative association between diurnal cortisol production (AUCg) at the admission time and the differences in diurnal cortisol production (AUCg) between T0 and T3 (r_s= -.76, p = .000), meaning that children who were producing more cortisol at the admission (AUCg) were more likely to diminish their cortisol production (AUCg) across time spent in the institution.

Also, child's ability to regulate cortisol at the admission was significantly and negatively associated with the differences in the child's ability to regulate cortisol between T0 and T3 (r_s= -.73, p = .000), meaning that children with less ability to regulate cortisol at the admission tended to augment their ability to do that, across time spent in the institution (Table 82).

Table 82

Correlations between AUCg levels, and Cortisol regulation and cortisol production and regulation differences between T0 and T3

	AUCg differences between T0 and T3	Cortisol regulation differences between T0 and T3
AUCg at T0 (admission) (n = 31)	-.76**	-
Cortisol regulation at T0 (admission) (n = 31)	-	-.73**

Note. Spearman correlations; **p < .01

3.8. The role of the quality of the institutional context in the child's cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation during institutionalization

The quality of the institutional context was assessed in terms of the institutional human resources, equipment and material resources, basic needs routines, developmental activities promoted and caregiving stability, and tested in association with the cortisol production and regulation throughout time spent in the institution, as follows (Table 83).

Association between institutional context variables and cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T1

Table 83

Correlations between institutional quality context and Diurnal cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T1

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Human Resources	.09	.28	.24	.30	.08
Equipment and material resources	.11	-.14	-.13	-.16	.10
Basic Needs Routines	.10	-.12	.24	.01	-.07
Developmental activities	.15	.12	.01	.06	.12
Caregiving stability	-.04	.07	-.08	.02	.07

Note. Spearman correlations

No significant associations were noted between the quality of the institutional context and the child's cortisol production and regulation at T1.

Association between institutional context variables and cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T2

Table 84

Correlations between institutional quality context and Diurnal cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T2

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Human Resources	-.47**	-.13	-.37*	-.45*	-.29
Equipment and material resources	-.05	.13	.01	.02	-.06
Basic Needs Routines	-.18	.11	-.25	-.17	.003
Developmental activities	.13	.000	-.02	.13	.17
Caregiving stability	-.02	-.21	.03	-.11	-.08

Note. Spearman correlations; **$p < .01$; * $p < .05$

At T2, significant and negative associations were noted as showed in table 84. Children in institutions with more human resources were more likely to show less morning (r_s= -.47, p = .008) and afternoon cortisol levels (r_s= -.37, p = .043), and also less diurnal cortisol production (AUCg) (r_s= -.45, p = .012).

Association between institutional context variables and cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T3

At the third moment of assessment a single significant and positive association was noted between the caregiving stability and the diurnal cortisol production (r_s= .42, p = .020), suggesting that children exposed to higher caregiving stability tended to show higher diurnal cortisol production (AUCg) 8 months after being admitted at the institution (Table 85).

Table 85

Correlations between institutional quality context and Diurnal cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T3

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Human Resources	.19	.21	-.03	.31	.07
Equipment and material resources	.12	-.11	-.11	.02	.08
Basic Needs Routines	.12	-.30	.04	.05	.03
Developmental activities	.23	-.05	-.02	.18	.18
Caregiving stability	.33	.28	.16	.42*	.17

Note. Spearman correlations; * $p < .05$

3.9. The role of the quality of the institutional context in the differences in cortisol production and regulation between T0 and T3

Variables of the institutional quality context were also tested in association with the differences between T0 and T3 in diurnal cortisol production (AUCg) and cortisol regulation. No significant associations were found (Table 86).

Table 86

Correlations between institutional quality context and cortisol production and regulation differences between T0 and T3

	AUCg differences between T0 and T3	Cortisol regulation differences between T0 and T3
Human Resources	.24	.05
Equipment and material resources	-.13	.03
Basic Needs Routines	.02	-.11
Developmental activities	-.02	-.002
Caregiving stability	.18	.23

Note. Spearman correlations

3.10. The role of the caregiver's sensitivity and cooperation behaviors in the child's cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation during institutionalization

Caregiver's sensitivity and cooperation behaviors were tested in association with the child's cortisol production and regulation at T1, T2 and T3.

Association between caregiver's sensitivity and cooperation and cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T1

No significant associations were noted between caregiver's sensitivity and cooperation behaviors and cortisol levels and regulation at T1 (Table 87).

Table 87

Correlations between caregiver's sensitivity and cooperation and Diurnal cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T1

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Sensitivity (n = 28)	-.08	.11	.03	.02	-.06
Cooperation (n = 28)	-.16	.01	.18	-.05	-.20

Note. Spearman correlations

Association between caregiver's sensitivity and cooperation and cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T2

At T2 a single marginally significant association was noted between caregiver's sensitivity behaviors and child's cortisol production at noon hours (r_s= -.31, p = .087), suggesting that children who were cared by more sensitive caregivers tended to show less cortisol production at noon hours (Table 88).

Table 88

Correlations between caregiver's sensitivity and cooperation and Diurnal cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T2

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Sensitivity (n = 31)	-.11	-.31†	-.28	-.25	-.06
Cooperation (n = 30)	.12	-.27	-.10	-.02	.15

Note. Spearman correlations; $^{\dagger}p < .10$

Association between caregiver's sensitivity and cooperation and cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T3

Also a single marginally significant association emerged at T3, between caregiver's sensitivity and cortisol regulation (r_s=.32, p = .080), meaning that children cared by more sensitive caregivers tended to show more ability to regulate cortisol across the day, eight months after being institutionalized (Table 89).

Table 89

Correlations between caregiver's sensitivity and cooperation and Diurnal cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation at T3

	Morning Cortisol	Noon Cortisol	Afternoon Cortisol	AUCg	Cortisol Regulation
Sensitivity (n = 30)	.26	-.30	-.23	-.12	.32†
Cooperation (n = 30)	.31	-.24	-.06	-.002	.29

Note. Spearman correlations; †$p < .10$

3.11. The role of the caregiver's sensitivity and cooperation behaviors in the differences in cortisol production and regulation between T0 and T3

Caregiver's sensitivity and cooperation behaviors were also associated with the differences between T0 and T3 in diurnal cortisol production (AUCg) and cortisol regulation, as follows.

Table 90

Correlations between caregiver's sensitivity and cooperation and cortisol production and regulation differences between T0 and T3

	AUCg differences between T0 and T3	Cortisol decrease across day differences between T0 and T3
Sensitivity T0 (n = 25)	-.05	.14
Cooperation T0 (n = 25)	-.40*	.05
Sensitivity T1 (n = 28)	.10	-.03
Cooperation T1 (n = 28)	-.10	.07
Sensitivity T2 (n = 31)	.13	-.05
Cooperation T2 (n = 30)	.08	.12
Sensitivity T3 (n = 30)	-.07	.13
Cooperation T3 (n = 30)	-.21	.08

Note. Spearman correlations; * $p < .05$

A single significant association was noted between caregiver's cooperation behaviors at T0 and differences in diurnal cortisol production between T0 and T3 (r_s= -.40, p = .045), suggesting that children who were cared by more cooperative caregiver's at the admission tended to diminish their cortisol production (AUCg) across time spent in the institution (Table 90).

4. RELATION BETWEEN NEUROENDOCRINE FUNCTIONING AND PHYSICAL GROWTH AND MENTAL DEVELOPMENT

4.1. The role of the child's cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation during institutionalization in the child's physical growth

Associations between cortisol production and regulation and child's physical growth across time were also analyzed.

Table 91

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and physical growth at T0

	Height	Weight	Head Circumference
Morning Cortisol	.12	.06	-.04
Noon Cortisol	.52**	.39*	.41*
Afternoon Cortisol	.45*	.37*	.25
AUCg	.51**	.42*	.29
Cortisol Regulation	-.03	-.07	-.16

Note: *Spearman* correlation; ** $p < .01$; * $p < .05$.

Table 91 shows that children producing higher levels of noon cortisol at the admission tended to be the ones with higher height ($r_s = .52$, $p = .003$), weight ($r_s = .39$, $p = .033$), and head circumference ($r_s = .41$, $p = .023$) at the admission. Also, children producing higher levels of afternoon cortisol and higher overall diurnal production (AUCg) were more likely to be the ones with higher height ($r_s = .45$, $p = .011$) ($r_s = .51$, $p = .003$) and weight ($r_s = .37$, $p = .038$) ($r_s = .42$, $p = .017$) at the admission.

Table 92

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and physical growth at T1

	Height	Weight	Head Circumference
Morning Cortisol	.08	.02	.12
Noon Cortisol	-.03	.18	-.01
Afternoon Cortisol	.08	-.06	.13
AUCg	-.04	-.11	.06
Cortisol Regulation	.16	.16	.17

Note: *Spearman* correlation

There were no associations at T1 between cortisol variables and child's physical condition (Table 92).

Table 93

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and physical growth at T2

	Height	Weight	Head Circumference
Morning Cortisol	.35	.28	.17
Noon Cortisol	-.02	-.15	-.23
Afternoon Cortisol	.27	.26	.31
AUCg	.28	.16	.12
Cortisol Regulation	.22	.15	.07

Note: *Spearman* correlation

Analyses revealed no associations at T2 between cortisol variables and child's physical condition (Table 93).

Table 94

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and physical growth at T3

	Height	Weight	Head Circumference
Morning Cortisol	-.08	-.07	.01
Noon Cortisol	.05	-.21	-.28
Afternoon Cortisol	-.15	-.21	-.23
AUCg	-.08	-.25	-.23
Cortisol Regulation	-.02	.06	.13

Note: *Spearman* correlation

No significant associations were found at T3 between cortisol variables and child's physical condition (Table 94).

4.2. The role of the child's cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation during institutionalization in the child's mental development

Associations between cortisol production and regulation and child's mental development across time were also analyzed.

Table 95

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and mental development at T0

	Cognitive	Language	Motor
Morning Cortisol	-.06	-.24	-.21
Noon Cortisol	.11	-.13	-.03
Afternoon Cortisol	.23	-.15	-.06
AUCg	.17	-.20	-.11
Cortisol Regulation	-.09	-.26	-.21

Note: *Spearman* correlation

There were no significant associations at T0 between cortisol production and regulation and mental development, in terms of cognitive, language and motor functioning (Table 95).

Table 96

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and mental development at T1

	Cognitive	Language	Motor
Morning Cortisol	.04	-.03	.12
Noon Cortisol	-.12	.28	.11
Afternoon Cortisol	-.14	-.31	-.06
AUCg	-.03	.06	.20
Cortisol Regulation	.03	.02	-.03

Note: *Spearman* correlation

No significant associations were found at T1 between cortisol production and regulation and mental development, in terms of cognitive, language and motor functioning (Table 96).

Table 97

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and mental development at T2

	Cognitive	Language	Motor
Morning Cortisol	.25	-.16	.27
Noon Cortisol	-.19	-.10	-.08
Afternoon Cortisol	.18	-.12	.15
AUCg	.22	-.05	.23
Cortisol Regulation	.08	-.16	.20

Note: *Spearman* correlation

Analyses revealed no significant associations at T2 between cortisol production and regulation and mental development, in terms of cognitive, language and motor functioning (Table 97).

Table 98

Correlations between cortisol production (morning, noon and afternoon levels and AUCg) and cortisol regulation and mental development at T3

	Cognitive	Language	Motor
Morning Cortisol	-.18	-.19	-.08
Noon Cortisol	-.01	-.18	.22
Afternoon Cortisol	-.20	-.19	-.12
AUCg	-.23	-.24	.03
Cortisol Regulation	-.08	-.09	-.06

Note: *Spearman* correlation

There were no significant associations at T3 between cortisol production and regulation and mental development, in terms of cognitive, language and motor functioning (Table 98).

The results presented above will be discussed in the next chapter of the present dissertation.

CHAPTER 4

PHYSICAL GROWTH, MENTAL DEVELOPMENT AND NEURO-ENDOCRINE FUNCTIONING IN PORTUGUESE INSTITUTIONALIZED CHILDREN: A LONGITUDINAL STUDY.

DISCUSSION & CONCLUSION

The results presented in Chapter 3 will be discussed below. The physical growth outcomes will be the first focus of this Chapter, followed by the results regarding mental development and neuroendocrine functioning, which will also be discussed.

Subsequently, limitations of the current study, suggestions for future research and practice, social and policy implications of the current study will be addressed.

1. DISCUSSION

1.1. PHYSICAL GROWTH

Evolution across time

One of the major goals of this study was to examine the evolution of the physical growth of children in Portuguese institutions, from their admission at the institution onwards.

The results obtained revealed a distinct effect of institutionalization for height, weight and head circumference. While for height scores we found no increase across time, for weight and head circumference significant gains were noted between T0 and T3. Unexpectedly, recovery was almost complete for head circumference at T1, when head circumference z score was close to 0, and complete at T3. For weight, only minor delays were noted 8 months after admission, while modest delays were noted for height across time. The minor to modest delays for weight and height, respectively, and the total recovery for head circumference, could be attributed to the fairly adequate diets that were administered in Portuguese institutions, that seem to correspond to the second category of deprivation, according to Gunnar's classification (2001). According to this three-level hierarchy, institutions classified in the second level meet the nutritional and health needs of the children, but fail to provide adequate stimulation and relational care. This level of deprivation could lead to developmental delays, but not to a severe growth failure. The fact that, across 8 months living in the institution, only minor delays were showed in weight and a total recovery was noted for head circumference might corroborate this hypothesis. The status of the head circumference at the admission was

better than to the other physical parameters, and this can also contribute to an almost complete recovery at T1. We also hypothesized that delays would be more pronounced as the length of institutionalization increases, which in fact was not observed. Findings suggesting this association came from studies with post-institutionalized adopted children, for whom a drastic change in the rearing context had occurred. In our sample, the absence of such observations relating length of institutionalization to more pronounced physical delays could be due to the fact that the children were still living under the adverse rearing conditions. The recovery for weight was more pronounced comparing to height, which is consistent with evidence that weight growth is more dependent on recent food intake than height. Indeed, we observed significant gains in weight immediately after the admission at the institution (within 2 months). On the other hand, height growth is more dependent on bone growth, which takes longer to occur (Miller, 2005). Given that a critical period for bone growth seems to occur early in life, growth retardation in institutionalized infants at critical periods of early development can compromise the growth trajectory (Cooper et al., 1997). It is also possible that height is more susceptible to some features of the institutional context comparing to weight and head circumference, thereby being more compromised (Dobrova-Krol et al., 2008). Our results also revealed a great variability in physical growth between T0 and T3, for all the three domains. Specifically for height, we observed gains (albeit in different magnitudes), for almost 55% of the sample. Also, in a recent study where a subsample of our sample of Portuguese institutionalized children was assessed both at the institution and later, when they were adopted, revealed marginal gains in height after about 5 months of integration in a supportive and stimulant environment within the adoptive family (Baptista, 2011). This result evokes the psychosocial short stature hypothesis inasmuch children tend to show immediately gains when removed from the depriving context and that the delays while institutionalized could be due to inadequate social and emotional care (Johnson, 2000a, 2000b). It is possible, and likely, that, even in the same institutional context, children do not experience the same level of deprivation. Nevertheless, despite the drastic change in the environment within the adoptive care, height remained the most deficient domain of physical growth (Baptista, 2011), which can indicate that some genetic characteristics, not explored in this study, could be underpinning this effect (Lettre et al., 2008; Weedon et al., 2007). Also, we should be cautious about the individual nutritional needs of children, since they vary depending on birth weight and whether preexisting deficits

exist (Johnson et al., 2010). Not only nutrition is likely affected by institutionalization but also, at the hormonal level, the growth factors secretion. However, both were not possible to explore in the present study. Psychosocial short stature can also be characterized, in part, by deficits in the production of endogenous growth factors, and thus recovery implies also reconstitution of normal growth hormone production (Johnson, 2000b; Johnson et al., 2010).

Quality of early care before institutionalization

Prenatal care

Prenatal risk and especially birth weight are proxy measures of the gestational environment and care that the child experienced during prenatal life. Birth weight reflects the supply of nutrients and oxygen to the fetus during the prenatal period (Cooper et al., 1997), but it can also be determined by maternal characteristics such as parity, smoking and social class (Goldstein, 1981, in Cooper et al., 1997). Although skeletal growth is certainly dependent on genetic factors, evidence confirms that growth is also responsive to environmental conditions, even within prenatal life. Negligence and/or environmental insults (e.g., malnutrition) experienced in critical periods during the prenatal and early postnatal life may result in lasting and permanent alterations in the growth trajectory (Barker, 1994, in Cooper et al., 1997). If malnutrition occurs early in life, the earlier the occurrence the more likely it will produce irreversible effects on individual's height and weight (Barker et al., 1993). In our sample, results indicated (through the measurements of birth weight and prenatal risk) that better fetal environment potentiated growth later in life, when children were living in the institution. This means to our understanding that less prenatal adversity and higher birth weight can buffer the influence of institutional rearing on physical growth. Curiously, prenatal risk and birth weight were not associated with the magnitude of gains/losses throughout the 8 months of institutionalization, except for the gains/losses in height. According to Cooper and colleagues (1997), skeletal size tracks from early life but this is not true for adult body mass index (a proxy measure for human body fat), which correlated with the existence, in our study, of an association between birth weight and gains in height (but not with gains in weight). Also, these associations between birth weight and height across time (T0, T1, T2, and T3) were stronger than the ones between

birth weight and weight across time. To the children who grew more in height during the 8 months of institutionalization, prenatal care seemed to be the poorest, since they had the lowest birth weight. It is possible that, for these children, the change in the rearing context (transition from family to institutional context) potentiated their growth in height, taking into account that height at admission was delayed and assuming that institutional care somehow managed to overcome the impact of birth weight on height growth, at least for some children. Regarding weight and head circumference, gains and losses during the 8 months in these two domains seemed to be more independent from prenatal life. In fact, as we shall see below, it seems that there is also greater susceptibility of height growth gains across the 8 months regarding the quality of the institutional context, which was not the case for both weight and head circumference gains.

Family-relational care

In regard to the family-relational care that children experienced before institutionalization and after birth, no associations were observed between the two composite measures and physical growth. Although we did expect an association between family-rearing conditions and ulterior development at the institution, we also acknowledge that the child's case files concerning postnatal life might often be incomplete, or at least the information be less accurate, when comparing to prenatal life, since the last one originates from individual health files, while social and family information is more difficult to obtain. It is our belief that the reliability of the information could be here interfering in the results. However, using the child's physical status at the admission at the institution as an indicator of the quality experienced within the context from where they were withdrawn, we found significant associations that we think deserve attention. Higher height, weight and head circumference at the admission were strongly associated with higher height, weight and head circumference (respectively) throughout institutionalization, meaning that the children admitted in better physical conditions tend to be the ones with better physical condition across time, but also the ones who show less gains and experience losses across time (which doesn't necessarily mean a significant decrease below their initial status). Nevertheless, these results point to a differential impact of institutionalization depending on the child's

initial status, emphasizing the fact that children who were more penalized by the caregiving context prior to institutionalization, from where they were withdrawn, were those who benefited most from the change to an institutional context. The inverse was also true, and may point to concerning results, since children who showed better results while living outside of the institution (although in a context that was considered inadequate to properly provide care) were the ones who were more penalized by institutionalization. These results seem to evoke the differential susceptibility hypothesis (e.g., Belsky and Pluess, 2009) which argues that "those putatively "vulnerable" individuals most adversely affected by many kinds of stressors may be the very same ones who reap the most benefit from environmental support and enrichment including the absence of adversity" (Belsky and Pluess, 2009, p. 886). Although a mechanism of differential susceptibility may be operating here, in our view, and knowing that institutional context is far from the expectable environmental, it is also possible that for some children, especially the ones more affected by prior adversity, the institutional context acts as an enriched environment, while for the ones less compromised at the admission, the institutional context may not differ much from the familial context, or be even worse than from where they were removed.

Quality of institutional and relational care

The quality of institutional care, in terms of their human resources, revealed to be associated with the magnitude of gains in height between institutional placement and 8 months later. Children reared in institutions with more human resources, showed stronger gains in height across the time spent in the institution. This indicates that the variability found in height growth, despite being highly dependent on genetic and prenatal factors (Lettre et al., 2008; Weedon et al., 2007), is also vulnerable to the environment. Care provided in institutions is commonly characterized as benign neglect because of the caregiver's unavailability to interact with the child, mostly due to the high child to caregiver ratios, high turnover shifts of multiple caregivers and highly challenging working conditions. Growth failure in institutionalized children closely resembles the characteristics of the psychosocial short stature (e.g., Blizzard 1990; Johnson, 2000a, b) that has been described as a condition of growth delay associated with prolonged child's exposure to social and emotional negligence by the child's

caregiver(s). Thus, our results suggest that more human resources available in the institution, indicating that more adults are likely to be available to interact with the child, could minimize the risk of the child experiencing benign neglect, which would decrease the threat on height growth. Johnson and colleagues (2010) found that the quality of the child-caregiver dyadic interaction has proved to be a predictor of child's physical recovery when children were integrated in foster care, but not while they were institutionalized (Johnson et al., 2010), although an intervention based on improving social-emotional-relationship in Baby Homes in Russia proved to influence and improve children's physical development (The St. Petersburg-USA Orphanage Research Team, 2009). In our study, caregiver's sensitivity measured at T2 was associated with the magnitude of gains in child's head circumference throughout institutionalization, but not with weight and height gains. Some authors argue that delays in head circumference growth are more related to psychosocial deprivation than sub-nutrition, since they found more long-lasting effects of psychosocial deprivation than of sub-nutrition in head circumference of children adopted from institutions in China (Cohen, Lojkasek, Zadeh, Pugliese, and Kiefer, 2008). In this sense, our results find considerable support in that they are influenced by the quality of relational care. Head circumference gains were only associated in our sample with the child's head circumference status at admission, and with the caregiver's sensitivity. However, it is not possible to determine here the extent to which gains in head circumference were mainly due to nutrition or psychosocial circumstances (e.g., Beckett et al, 2006). Nevertheless, it is important to note that gains are associated with the specific aspect of relational care with the caregiver and not with the macro dimension of institutional care (e.g., human resources, equipment and material resources, basic needs routines, developmental activities and caregiving stability), which seems an important contribution to the field.

Age at admission

Children admitted earlier at the institution (< 12 months) were significantly smaller 5 and 8 months later than children admitted above 12 months, who were close to full recovery 8 months after the admission. Children admitted earlier showed a decrease in height z score between T1 and T2, while children admitted older grew between T1 and T3. As seen in other studies (Castle et al., 1999), we can argue that the quality of

care provided in the institutions are less suitable for younger children needs, since they can do little to trigger effects on the environment without the contribution and support of caregivers (Gunnar, 2001). Indeed, our results also revealed that children admitted earlier were reared in institutions where there was less equipment and material resources, less developmental activities were promoted and less sensitivity and cooperation were experienced in interaction with the caregivers, which may have contributed in a relevant way to a loss of height z scores in younger infants, while older children showed improvements. Recovery in older children may also be related to an increasing capacity for adaptation, due to the likely emergence in older children of certain protective factors allowing them to cope with the growth-depriving context. Indeed, growth seems to be age-dependent, and it is possible that children admitted earlier will show gains throughout their lives. During the rapid growth phase between 0 to 18 months, children are more vulnerable to nutritional deficits, while after that phase nutritional intake is less crucial to growth rate (Johnson, 2000a; Johnson et al., 2010). The study of Dobrova-Krol and colleagues (2008) corroborate this hypothesis, since they found, in their sample of Ukrainian children institutionalized very early in life, a tendency for physical growth recovery after the age of 24 months, even in the absence of alterations in the depriving context. This allied to the fact that during this period infants can do little to evoke care, lead us to believe that younger infants in depriving contexts are highly susceptible to insufficient intake, thereby presenting slower growth rates. Also, we should consider the child's protective mechanisms emerging later in life to understand determinants of recovery, as are the growing capacities to actively shape the environment, rather than look only to the environment. Time is also important in what concerns the quality of care provided during the prenatal life, since it takes months to recover from adverse experiences during this period (Minde, 2000). In fact, the two groups didn't differ in terms of prenatal risk experienced in birth weight and also in terms of the early family risk, which lead us to believe that differences at admission may be related to age itself. Children admitted earlier also showed less weight across time, except 8 months later when the two age groups were similar. For children admitted at older ages, stability in weight was noted, showing values within the normative range across time, while children admitted earlier gained significantly across time, showing just a small delay 8 months after the admission. This is to say that the two groups converged 8 months after institutionalization, despite the inferior status at admission of younger children, which we believe was due to nutritional deficiencies

while living with the family, since they had not experienced additional prenatal or early family risks, and also they did not weight less at birth than children admitted older. As we already noted, weight is much more dependent on recent food intake than height, allowing children to recover faster (Miller, 2005). Head circumference was similar for both groups across time. However, children admitted older showed stable normative values across time while children admitted earlier started with a modest delay ending up showing only a small delay, after 8 months of institutionalization. The fact that head circumference growth is more easily influenced before 12 months of age than later, when effectively it takes longer to change (Johnson, 2007, personal communication – in The St. Petersburg-USA Orphanage Research Team, 2008, p. 139) may help us explain the initial differences which, although not statistically significant, seem clinically relevant if considering that children admitted older were at the normative range since the beginning and children admitted earlier showed moderate delays. It is interesting that for children admitted above 12 months, in the domains where they were within the normative range at admission (weight and head circumference), no significant gains occurred during institutionalization, which led us to hypothesize that institutional care cannot improve the child's physical condition when there is less room for recovery. This is consistent with our results revealing that children admitted in the worst conditions tend to be the ones who recovered most, while the ones who entered the institution with better physical status tend to show less gains or even losses across time.

1.2. MENTAL DEVELOPMENT

Evolution across time

Mental development also revealed to be affected by the experience of institutionalization, since at the end of the 8 months moderate deficits were noted for cognitive, language and motor development. This is consistent with the extant international literature that frequently noted developmental delays in cognition, language and motor development for institutionalized (Vorria et al., 2003; Zeanah, Smyke, Koga, Carlson, & the BEIP Core Group, 2005) and post-institutionalized children at the time of the child's integration in the adoptive family, regarding children adopted from institutions around the world (Albers et al., 1997; Ames, 1997; Cohen et al., 2008; Miller, Chan, Comfort, and Tirella, 2005; Rutter & The Era Study Team,

1998). These delays are often related to under-stimulation and unresponsive care provided while under institutional care. In fact, when children from institutions were compared with children raised in foster families, in which a more normative family-type rearing arrangement is offered, better cognitive results were noted for the foster children and a continuing "cost" emerged for children that remained institutionalized (Nelson et al., 2007). Despite that, gains of approximately 10 percentage points were noted for language and motor functioning, after eight months of institutionalization. For cognitive development no gains were found, which may be attributable to the fact that this was the area of mental development that was less delayed at the baseline, leaving less room for extra development in a context that is naturally far from the expectable. As we expected, effects of institutionalization in Portugal were not so deleterious, and this may be due to the fact that the levels of deprivations in Portuguese institutions are not so global and severe. Nevertheless, we observed that children in institutions needed a considerable amount of time to interact and establish rapport with the researcher who was administering the scales of mental development and only few showed signs of joy and interest in the procedure. It was also very common for children to give up the task easily and quickly show signs of hyperactivity and difficulty in maintaining attention. We suspect that both factors may account for the observed delays in our sample. Length of institutionalization doesn't appear related to mental delays, as was our expectation based on studies with adopted children from institutions. Similarly to our explanation for physical growth, we thought that the fact that our sample remained institutionalized and living under adverse conditions did not allow this association to emerge, while in the context of adoption it would be likely that the more delayed children would be the ones that spent more time of their lives in the institution.

Quality of early care before institutionalization

Prenatal care

The results from our study were consistent with our expectations, as they revealed significant associations between birth weight and cognitive, language and motor development, and also between prenatal risk and the variables of mental development. Children with low birth weight (LBW) consistently showed reduced IQs in comparison with children who were born with normal weight at term (Bhuta, Cleves, Casey, Cradock, & Arnand, 2002; Hille et al., 2001). Indeed, in our sample, at least at

T1 and T2, it was evident that children with less birth weight (which doesn't imply being born LBW; LBW < 2500gr) are more likely to show worse cognitive functioning. Moreover, children showing the worst language and motor performances at T2 and T3 were more likely to have been born with low weight. Also prenatal risk emerged associated with cognitive development, but only at T3. We recall here that prenatal risk is a composite measure comprising the following risk factors: maternal physical disease (e.g. AIDS, Hepatitis), maternal substance abuse during pregnancy, pregnancy without medical surveillance, and prematurity. It is our belief that prenatal risk mirrors a framework of high maternal stress experienced during pregnancy, which in turn have also been found in association.

Although fetal programming hypothesis have been increasingly suggested as a possible explanation for persistent effects of prenatal adversity in developmental outcomes, our results do not seem to point to a possible prenatal programming of mental development. As defined by Talge and colleagues (2007, p. 246) "Fetal programming is a concept that describes the fetus' physiological adaptation to the characteristics of the intrauterine environment within which it is developing. Such adaptation may subsequently affect the set points of physiological systems of the body undergoing rapid structural and functional changes, including those that maintain homeostasis. If not optimally suited for the postnatal environment, the prenatal physiological adaptations may render the offspring vulnerable to the development of health problems later in life." Evidence for a programming hypothesis concerning the effects of prenatal adversity on children's mental development failed to find support in our results because of the discontinuity found in the associations between birth weight and mental development in each moment of assessment. If such association was consistent across all time points of assessment, then we could speculate about a fetal programming effect, but this was not the case in our sample.

The status at admission in terms of language and motor development revealed to be positively associated with language and motor functioning at subsequent moments of assessment, respectively, showing that children entering the institution in a better condition tended to remain in the same condition across time. However, children who were admitted with better cognitive status tended to be the ones with better condition 2 months later, but not 5 and 8 months later. Cognitive development assessed 5 and 8 months after institutionalization was not associated with child's cognitive score at the

starting point. Therefore, the magnitude of gains for cognitive functioning while institutionalized was associated with the child's cognitive initial status, wherein children admitted in worst conditions tended to show higher improvements in their cognitive competence whereas children admitted in better conditions tended to gain less or even diminished their performance. The same was not true for language and motor development, since no significant association was found between the status at the admission and the magnitude of gains across time. Results regarding the role of the early family context risks for mental development revealed a marginally significant association between family-relational risk and cognitive development, suggesting that children who experienced less risk while reared in the family context were less negatively affected in their cognitive performance at the admission, immediately after the withdrawn from the family. It is possible that, despite the environmental risk, individualized care, in terms of knowledge about the child, sensitivity and availability for them, was more or less preserved where less risk existed, and thereby cognitive development was less impaired (Castle et al., 1999). No association between family-relational risk and cognitive development was found in the following moments, suggesting that the entry into the institutional context may have contributed to halt this association. In contrast, children who were exposed to high family-relational risk were less likely affected in terms of language performance, since they showed better performance on language tasks, both when admitted at the institution and 8 months later. This seems contradictory to us and our unique possible explanation relates to the fact that higher family-relational risk reflects chaotic rearing contexts where domestic violence is easy to occur, leading us to hypothesize that the child has developed language as a "survival" strategy, comparing to children in more preserving contexts. It is likely that these children have to protest and claim attention to their needs in face of the daily events, using verbal communication as a useful tool to achieve their goals. The fact that 8 months after the admission this association remained significant may reveal that somehow during the stay in the institution children used the verbal communication strategy to claim for attention, which is advantageous in a highly "competitive" rearing context.

Also, the magnitude of gains in language development was positively associated to the emotional-negligence risk. Emotional negligence risk was intended to capture the degree of unavailability of the maternal caregiver to establish a special and privileged

relationship with their children and to take care for him/her. It was interesting that the children who experienced higher unavailability during the life period in which they lived with their biological family, tended to benefit more from being institutionalized, in terms of language development. Language development is enhanced within a dyadic relationship and it is highly possible that children experiencing high emotional-negligence risk missed the chance to establish a special relationship within their family context, thereby affecting language development. In our view, this result may suggest that the availability of the caregivers has a significant role in language development.

Quality of institutional and relational care

It was interesting that human resources and the developmental activities promoted in the institution were the dimensions of the quality of the institutional care that were associated with language and motor development. In general, institutions have good equipment and material resources and meet basic needs routines, but these two indicators of the quality of the institutional care were not related to any of the dimensions of mental development. Rather, language development and motor development were found associated with human resources and developmental activities, respectively, which suggests the need of technical, educational and caregiver staff availability to interact with the infants in a stimulating way, over the equipment and materials. Children in institutions where more human resources exist and more developmental activities were promoted, tended to show better language and motor development, at least 5 months after institutionalization. We do not know how much of these contextual variables contributed to an increase in language and motor development over the contribution of the status at the admission. Nevertheless, these results are consistent and in line with the findings from the intervention in Russian Baby Homes that were organized in small groups of children with fewer caregivers assigned to each child, which suggested an increase in child's developmental scores when more caregivers were available to interact with the child. Intervention was even more effective when this structural changes were accompanied with caregivers' training to respond to children in a more warm, sensitive and caring way, especially during periods of play and while caregivers are performing basic routine duties (The St. Petersburg-USA Orphanage Research Team, 2008). Notwithstanding, our results do not mean that

children who were at best conditions 5 months after the admission, were the ones who benefited more from institutionalization. Indeed, our results also revealed that children showing better language development at admission tended to remain in the same level of functioning across time and the ones who revealed higher gains in language seemed to be children who were reared in institutions where less human resources existed. In this regard, our view is that children who entered institutions from less depriving families revealed to be more impaired in terms of language development and it is possible that they had to develop language as a survival strategy while in the institutional context, where children compete for attention. The ones from high risk families had to develop communication much earlier than institutionalization, due to the high risk family context. Also, institutions where there were more human resources may have been able to respond more promptly to the child's needs, giving the children less chance to claim for attention and to express their needs, and this could have resulted in less use of language and consequently less development.

We also found that the magnitude of gains occurring in cognitive functioning was negatively associated with the caregiving stability. This means that institutions where less caregiving stability was noted tended to be the ones where more gains in child's cognitive development occurred. In this respect, Castle and colleagues (1999) found that cognitive development was affected by the quality of individualized care, and our results, although unexpected, do not discard this explanation, since higher caregiving instability does not mean less individualized care provided to each child, in terms of sensitivity and availability for them. In fact, higher caregiving instability, in the sense of many different caregivers taking care of a child (opposing to a fixed number), can indeed derive from higher caregivers' number and availability, and according to some authors many different caregivers could promote cognition in a short period of time (The St. Petersburg-USA Orphanage Research Team, 2008).

Higher quality of the relational care, in the form of sensitive caregiving, stimulation of development, positive regard for the child, positive affect and attachment, have been associated with higher levels of DQ (Developmental Quotient) in institutionalized children (Smyke et al., 2007). Our results from correlational analyses between caregiver's sensitive and cooperative behaviors towards the child and their mental development were not congruent with the findings above. Higher caregiver's cooperation, 2 months after admission at the institution, was associated with less

language development, and after 5 months from the admission, higher caregiver's cooperation and sensitivity were associated with less cognitive development. In addition to these marginally significant associations, no more associations were found in the other moments of assessment. That is, in our sample, we found lack of support for the expected association between child's mental development and quality of relational care in terms of sensitivity and cooperation experienced in relation with the caregiver of reference. We suspect that a limitation to our procedure has to be with the fact that we assessed caregiver's sensitivity and cooperation from a privileged moment of interaction with the child, in which caregiver was totally free to be dedicated to that child, rather than a more naturalistic interaction where caregiver has to deal and care for a group. It is likely that caregivers were not so sensitive and cooperative on a daily basis, when they have to take care of many children at the same time. Also, we measured sensitive and cooperative behaviors of only one caregiver that interacts with the child, having no information about the sensitive and cooperative behaviors of other caregivers towards that child that can equally have an effect on his/her mental development. Additionally, the caregiver selected to be videotaped in interaction with the child did not have a special relationship with him/her in most cases, especially when the child was new in the institution or was living there not for long. Until T2 assessment, it was difficult to identify a caregiver of reference, because the time to start establishing a relationship was too short or even because children were generally too young to show specific behaviors toward a single caregiver.

Age at admission

After 8 months of institutionalization, children admitted earlier than 12 months of age were more cognitively impaired than children admitted older than 12 months. In fact, children admitted at an older age showed a pattern of increase after T1 assessment, achieving the 45th percentile in the cognitive scale at T3 suggesting that an almost normative functioning was achieved 8 months after initial placement. Conversely, children admitted at earlier ages developed a trajectory of losses from T1 up until T3, when they were even more impaired than at the admission moment. In what concerns the language development, this was not significantly different across time depending on the age of admission at the institution, although it seems important to note that children

admitted older than 12 months recovered across time and achieved the 39th percentile after the 8 months spent in the institution, while children admitted earlier showed a decrease in their language competence initially, and gained afterwards but only reached the 25th percentile. Although differences between groups were not statistically significant, the recovery for the younger group was notoriously insufficient comparing to the 39th percentile reached by the older group, especially when considering that they had a very similar starting point. For motor development, differences between groups were evident as they emerged immediately after placement, that is 2 months after institutionalization, and persisted up until 8 months of institutionalization. Children admitted older than 12 months showed significantly better motor development 2, 5 and 8 months after initial placement, recovering significantly across time up until the 56th percentile. In contrast, children admitted earlier showed a slight recovery, achieving only the 29th percentile. Overall, children admitted earlier than 12 months were more delayed and impaired in their mental development than children who entered the institution at older ages. These results are consistent with what we expected, and are in accordance with some international literature that advocates that the quality of the institutional care is much far from the expectable for children of younger ages, wherein older children had access to much more toys and diverse physical experiences (Castle et al., 1999). Not only because younger children are less capable of eliciting care (Gunnar, 2001), but also because caregivers tend to confine them to their cribs or chairs/seats, depriving them from sensorial stimulation, especially because caregivers need free time to perform other institutional duties. This environmental under-stimulation of the younger children may have contributed to their worst motor and cognitive development, since cerebellum, a part of the brain that controls simple motor skills and mediates some specific cognitive processes, is highly susceptible to environmental conditions and may hinder normative development (Bauer, Hanson, Pierson, Davidson, and Pollak, 2009).

An interesting fact is that at the baseline assessment (T0), children of both groups (admitted earlier than 12 months *vs* older than 12 months of age) were quite similar in terms of cognitive, language and motor development, with no statistically significant differences noted between groups. This means that the establishment of an ascendant or descendent trajectory seems to have to do with the interaction between age and institutional rearing conditions. In fact, analyses of differences between the two age groups contributed in an interesting way to unravel the differences found in mental

development. Results revealed that children admitted earlier at the institution (< 12 months) did not experience more prenatal risk or showed less birth weight than the ones admitted later (≥ 12 months). Also, children admitted earlier were not exposed to higher family-relational risk or even higher emotional-negligence risk. Rather, children admitted earlier than 12 months were reared in institutions where less equipment and material resources existed and less developmental activities were promoted by the institutional staff, and also they were cared by less sensitive and cooperative caregivers at some time points during the stay. This is of great importance as it revealed that the quality of institutional and relational care really matters and corroborated the idea of other researchers (Castle et al., 1999) when they state that institutional placement is even less adequate for young children.

1.3. NEUROENDOCRINE FUNCTIONING: CORTISOL PRODUCTION AND REGULATION

Evolution across time

Contrary to what was our expectation, children showed a typical pattern of daily cortisol production, in every moment of assessment, with a peak in the morning hours and a decrease across the day, especially between morning and noon hours. Therefore, we did not corroborate the findings from Carlson and Earls (1997) and Kouprina and colleagues (1997, in Tarullo & Gunnar, 2006) studies, which reported a flattened pattern across the day, without a peak in the morning and no systematic decrease across the day. Our results, especially from physical growth, already pointed to fairly adequate institutional arrangements for Portuguese institutionalized children, as noted in the recovery in weight and head circumference growth. Thus, it is possible that the quality of the institutional care was not so bad to cause dysregulation of the daily cortisol rhythm. It is also of note that it is possible that the stress regulation system is more robust than other systems underlying cognitive, language and motor development for example, which were more detrimentally affected across institutionalization, as was also seen in other studies (van den Dries, Juffer, van Ijzendoorn, & Bakermans-Kranenberg, 2010).

As we were expecting cortisol levels were the highest at the admission moment. As we argued, entering the institution may be viewed as a stress-eliciting event, as children were withdrawn from their original and family context and at the admission they encounter many people whom they have never met and from whom they did not know what to expect (Cicchetti & Rogosch, 2001b; Gunnar & Donzella, 2002). Additionally, it is possible that elevated cortisol levels may also have resulted from grief experienced by children because of the separation from their family (Dozier, Peloso, Lewis, Laurenceau, & Levine 2008), and it is also likely that most of them were not used to live with so many children in the same place and in a highly competitive context. Nevertheless, our results point to a decrease in the diurnal cortisol levels at the moments after the admission, specifically at T1 and at T2, but an increase was noted at T3 with a tendency to reach the cortisol levels at admission. We speculate that the elevated levels at admission must be naturally followed by a decrease in the reactivity of the HPA axis, since they were likely a result of a stressful event. Also, it is likely that the institutional context may have revealed less stressful than the family-environment immediately following the admission, and that the HPA axis was somehow buffered and produced less cortisol. However, because the quality of the institutional care is far from the "expectable", this less reactive phase (between T1 and T2) was followed again by an increase in the cortisol levels that we suppose resulted from ongoing stress experienced at the institution, reflecting a dysregulation of the HPA axis which was no longer buffered by the care provided. In fact, we should not forget that cortisol increases when demands exceed the individual's coping resources (Dettling et al., 2000), which may be occurring here after a considerable time spent in the institutional context. We also found that, at T1, the cortisol decrease across the day was less accentuated than at T0 and at T3, but this may seem to have resulted from the more elevated peak at 7 am, in both moments of assessment, T0 and T3. This finding suggests that dysregulation of the rhythm may be protected by some plasticity of the neuroendocrine system (Cicchetti & Valentino, 2006). However, as was noted by Zeanah, Gunnar, McCall, Kreppner and Fox (2011), it may be possible that sleeper effects may be acting here and explaining the absence of a dysregulation of the rhythm, as "some apparent consequences of early institutionalization do not become apparent until many years after the children have been adopted out of the institution and reared in highly advantaged families, although this may depend on the severity of the early experience" (Zeanah et al, 2011, p. 157).

Quality of early care before institutionalization

Prenatal care

Experiencing adversity during fetal period could result in an elevated bio-behavioral reactivity to stress, with raised activity of the HPA system, which is thought to be a mediator of the effects of the prenatal exposure to stress (Phillips, 2007). In our study, associations were found between both measures of prenatal adversity (prenatal risk and birth weight) and cortisol levels at the admission at the institution. Some authors (Tarullo & Gunnar, 2006) suggested that the morning levels were the best diurnal measurement to reflect the degree of early adversity, and our results suggest that children who experienced higher adversity in prenatal life would be more susceptible to produce higher morning cortisol levels, being consistent with the idea of an elevated reactivity of the HPA system. Nevertheless, higher prenatal risk was also associated with less noon cortisol levels, which we are not able to explain although we suspect that noon levels in children that experienced more prenatal risk may be under more social regulation and be more buffered by the institutional care at the admission than morning levels. As stated by Gunnar and Quevedo (2008, p. 142) "Because the diurnal slope of cortisol is largely determined by the height of the early morning peak" our results also point to a better cortisol regulation, determined by the morning levels and from which afternoon levels were subtracted, in children with higher prenatal risk. It is our opinion that we should read this result in light of this explanation. Associations between birth weight and cortisol levels revealed that the higher the birth weight the higher the noon levels, which was coherent with the result from the negative association between prenatal risk and noon cortisol levels. It is expected that children experiencing more prenatal risk tended to be born weighing less, and since more prenatal risk was associated with less noon levels, it is logical that children with more birth weight (meaning less adversity during prenatal life) tended to produce more cortisol at noon hours. Birth weight was also positively associated with the overall production across the day (AUCg), suggesting that children who were born weighing more tended to produce higher cortisol levels during the diurnal hours when entering the institution. It is also true that children who were born weighing more tended to show better physical condition at the admission at the institution, which is also associated with higher cortisol production at the admission. It is our belief that children with better physical condition

more easily produce a cortisol response, since some physical robustness is needed to mount a cortisol response, as suggested by some authors (Cicchetti & Valentino, 2006). No associations were found in the following moments, which were also not seen as stress events.

Family-relational care

Contrary to what was our expectation, our results revealed that the quality of early care experienced within the biological family was not related to the levels of cortisol at the admission at the institution and during institutionalization. It is our expectation, according to some studies (van der Vegt et al., 2009), that the severity of the early maltreatment would be related to the basal cortisol levels and diurnal pattern, with higher levels and steeper slope associated with more severe experiences before institutionalization. However this was not the cause, and we advance that this may be related to the fact that most of our children were admitted at the institution because of some form of neglect, and very few because of physical and/or sexual abuse. A lack of range in the severity of early adversity experienced may be accounting for the absence of such an association. Also, as other authors suggested (Cicchetti and Rogosch, 2001b; Gunnar & Vazquez, 2001, 2006), neglect seems to have a minor effect on basal cortisol levels, and this can also be the reason why no association was noted between the quality of the early family-care and cortisol levels at the admission and following moments at the institution.

We were also interested in understanding the relation between the cortisol levels and diurnal production at the admission and the cortisol production in the following moments. What we found was that no association existed between the admission moment and the other assessments made during institutionalization, meaning that it is not possible to predict the cortisol production during the period of institutionalization from the admission status. This led us to hypothesize that the intra-individual variation in terms of the production in each moment of assessment may not be "programmed" by the admission status, which means that the fact that, at T3, levels increased close to T0 levels, were not due to increased production by the same children that produced higher levels at the admission. Indeed, children who produced higher overall diurnal cortisol at

the admission, tended to diminish their cortisol production between T0 and T3, while children who produced less when admitted at the institution tended to increase their production across time. This means that the effect of institutionalization was differential depending on whether children produced more or less cortisol at the admission, meaning that institutionalization may have proved to be a buffer for some children but not for others. Since there was no relation between the early family context risks and the cortisol levels produced at the admission, we cannot attribute the higher production at the admission to the severity of adversity experienced before institutionalization. The only thing that we can assume is that children who were more physiological reactive to the withdrawn from the family and consequent admission at the institution tended to decrease their overall diurnal cortisol levels across time. The inverse was also true, for the ones who were less physiological reactive at the admission.

Quality of institutional and relational care

Our hypotheses suggested that some features of the institutional environment would be related to the child's cortisol levels, and indeed this happened to some extent. After 5 months of institutionalization, cortisol levels were found more susceptible to the quality of the institutional environment as they were associated with the number of institutional human resources. Children reared in institutions where more human resources existed tended to show less morning, afternoon and diurnal overall cortisol levels. We do know that HPA axis is under strong social regulation at early ages, and it is interesting that it is a "human" characteristic of the quality of institutional environment that is related to the diurnal cortisol production. We suggest that more human resources working at the institution would mean more availability from the caregivers to interact with the child and to provide care more adequately. This may reveal higher quality in the care provided acting as a buffer to potential daily stressors. Also, at T3, 8 months after the admission at the institution we found that the higher the caregiving stability, the higher the overall daily cortisol production. In fact, this result was the opposite of what we might have expected, since higher caregiving stability is supposed to mean better quality of care. However, as was the case for cognitive development, we argue that more caregiving stability doesn't mean more individualized care and more availability to interact with the child, which is crucial for cortisol

regulation (Dettling et al., 2000; Gunnar & Quevedo, 2007; Sroufe, 2000). Indeed, higher caregiving stability could derive from a pool of few caregivers available to work in the institution.

To some extent, the present results confirm that the caregiver's sensitivity and cooperation toward the child act as a buffer of the HPA axis. Children who experienced more caregiver's sensitivity produced less cortisol at noon hours after 5 months of institutionalization, and were also more able to diminish cortisol throughout the day after 8 months of institutionalization. Caregiver's cooperation at the child's admission at the institution was associated with a decrease in the overall cortisol production between T0 and T3. This means that children who were able to downregulate their HPA axis after a stress event, as was the admission moment at the institution, and throughout the 8 months of institutionalization, were cared by more cooperative caregivers when they were placed at the institution. It is of note that the caregiver's behavior towards the child at the admission was significantly related to their HPA axis regulation across time spent in the institution, and not caregiver's behaviors at other moments of assessment, which highlights the importance of the child's admission moment at the new life context.

Age at admission

Admission at the institution at 12 months of age or above, when it is expected that a decrease in the HPA axis responsivity to stressors occur, was not different from being admitted earlier, in terms of the cortisol levels produced and the diurnal cortisol regulation at the time of admission. This means to us that, even though some of our children may be under the Stress Hyporesponsive Period (SHRP), this doesn't mean less reactivity to the stress experienced at the admission at the institution. Both groups showed the highest levels of cortisol at the admission moment, meaning that both developed a stress response to having been withdrawn from their family. The SHRP is hypothesized to be maintained by caregiving behaviors, which in this case failed to buffer the stress response, which was expectable since children were no longer cared by their usual caregivers and were meeting their new caregivers at the entry into the institution, which *per se* may also constitute a stress event. Also, in the following moments of assessment, the two age groups didn´t show significantly different patterns

of cortisol production and regulation, which means that the effects of institutionalization were independent of the age of the child at the admission.

1.4. RELATION BETWEEN NEUROENDOCRINE FUNCTIONING AND PHYSICAL GROWTH AND MENTAL DEVELOPMENT

Cortisol production and regulation and Physical growth

Our results pointed to a somehow unexpected finding, with children in better physical condition at the admission at the institution producing higher levels of cortisol, and a lack of association between physical growth within the institutional context and production of cortisol over time. Some authors have suggested that the effects of early adversity on the HPA axis regulation may be one of the mechanisms that partly mediate the effects of adversity on physical development (Gunnar, 2000; Heim et al., 1997). Increased activity of the HPA axis, manifested by elevated levels of CRH and cortisol, is suggested to impact on the growth axis, through inhibition of growth hormone and growth factors under conditions of psychosocial deprivation and early neglect, consequently contributing to growth delays (Albanese et al., 1994; Cianfarani et al., 2002). Thus we would expect to find, in our sample, negative associations between child's physical growth and cortisol production, meaning that the children with higher cortisol levels were the ones more impaired in terms of physical growth. But this was not the case. Thus, at the admission, we found a positive association suggesting that the children producing higher cortisol levels were the ones admitted in better physical condition, which we are only able to analyze and explain based on the suggestion of Cicchetti and Valentino (2006) that some physical robustness is needed to mount a cortisol response to a stress eliciting event as was the admission at the institution. The fact that, in the following moments that were not as stressful as was the admission moment, no associations were found between cortisol levels and physical delays, led us to suggest that HPA axis in our sample was not significantly affected by the institutionalization and thus cortisol levels were not too high or too low, as in other samples. Accordingly, it is likely that the physical growth delays detected in our sample may be due to other reasons rather than high levels of cortisol production affecting growth axis.

Cortisol production and regulation and Mental development

Associations relating cortisol production and mental development in our sample failed to corroborate the suggestion that cognitive deficits in post- institutionalized children may have arisen from the exposure in early life to neurotoxic levels of glucocorticoids that resulted from the chronic activation of the HPA axis (Marshall & Kenney, 2009). It seems from our results that there was no chronic activation of the HPA axis in our sample, nor hypo and hyper-cortisolism. Thus, as a significant dysregulation of the HPA axis should not have occurred in our sample, it seems that this was not a plausible reason to some of the cognitive deficits that persisted throughout the 8 months of institutionalization. However, it does not mean that our sample was not affected, for example, on learning and memory capacities, since they are both mediated by the hippocampus which is thought to be more vulnerable to cortisol effects (Sapolsky et al., 2000). In fact, as we used a general measure of the cognitive development, it is possible that other measurements of specific cognitive functions might have revealed significant associations with cortisol production and regulation across time spent in the institution.

2. CONCLUSION

The present study aimed to describe the development of children placed in Portuguese institutions from the time of their admission onwards. It focused specifically in physical growth, mental and neuroendocrine development throughout the first eight months that children spent in the institutions. The study sought also to understand the contribution of several aspects of the institutional life for the child development, as well as aspects of child's life prior to institutionalization. Throughout the 8 months of institutionalization, children grew in weight to values that were close to the normative values expected for their age. Unexpectedly, they showed complete recovery in head circumference growth, contrarily to the suggestions from international studies (Johnson et al., 1992b; Johnson, 2000a,b; Rutter & The ERA study team, 1998; van Ijzendoorn et al., 2007). Inversely, height proved to be the physical domain that was more impaired and resistant to change, remaining at the same delayed level across the eight months of institutionalization. Children admitted at the institution at older ages were less impaired

at the admission and across time, while the scenario is more worrying for the younger children, especially in height growth since institutionalization accentuated their delay. Globally, institutionalization was beneficial for child's physical growth in weight and head circumference domains, especially for children admitted in worst physical conditions. Prenatal experiences proved to contribute in a significant way to the child's physical trajectory, with birth weight having an important role in the child's height recovery across time. However, the amount of human resources working at the institution was also important to height recovery, a result that we thought deserves best attention, as height was the most worrying domain of physical growth across time. Caregiving behaviors, especially their sensitivity towards child, also need to be considered an important contribution to the child's recovery in head circumference.

Globally, institutionalization was not beneficial to mental development, especially for cognitive development, as children did not improve their performance over time. Motor development was the less impaired domain. Nevertheless, if children were admitted above 12 months, their cognitive development, such as language and motor development, was less threatened, which was an important contribution to the field. Younger children are more deleteriously affected in their mental development by institutionalization. Institutionalization was more beneficial in terms of cognitive development for the children at worst cognitive status at the admission. Especially, cognitive and motor development was more susceptible to the prenatal life experiences, with less adversity during this life period constituting an ingredient for better cognitive and motor outcomes. Motor development was also susceptible to the developmental activities promoted at the institution and language development to the amount of human resources working in the institution. Contribution of the caregiver's behaviors in terms of their sensitivity and cooperation towards the child deserve more attention in further studies, as it was not clear their role in the child's mental development.

The HPA axis reactivity was the highest at the admission at the institution, a moment which *per se* was a stress-eliciting event, and decreased slightly in the months that followed, to then increase again after 8 months of institutionalization, when children were producing cortisol at levels close to the ones of the admission stressful moment. Buffering of the HPA axis was not so efficient after the 8 months of living in the continuously demanding institutional environment. The admission at the institution proved to be highly stressful, being able to elevate cortisol levels even during a

hypothesized stress hypo-responsive period. Children with higher physical robustness were more able to mount a cortisol response. Fetal and perinatal adversity were major contributors to the child's cortisol production at least until the admission moment, with no further associations across the 8 months of institutionalization. Children who benefit more from institutionalization by decreasing their overall diurnal cortisol production over time were the ones who were more reactive at the admission at the institution. Also, children who were more able to regulate their cortisol levels over time were the ones who regulated worst the cortisol levels at the admission. Some characteristics of the institutional environment somehow contributed to the cortisol levels produced, as was the case for the human resources working in the institution. Caregiver's sensitivity and cooperation were also important to the downregulation of the HPA axis across time. Both physical growth and mental development were not negatively affected by the levels of cortisol produced by children throughout the 8 months.

3. LIMITATIONS OF THE STUDY and FUTURE RESEARCH

Results of the present study are globally consistent with other studies in showing that institutionalization has negative effects on child development and is far from the expectable environment to promote their development. However, some limitations were found in the present study that should be acknowledged:

First of all, the small sample size and the absence of a normal distribution to the variables in use, that compromised the use of more complex and robust statistical analyses. This limitation did not allow us to draw conclusions that would have contributed to encourage further discussion on some of the results. Furthermore, due to the fact that some associations are only marginally significant, results should be cautiously interpreted and thoroughly analyzed in future studies.

Secondly, this is a short-term longitudinal study that followed the development of children for only 8 months, which in some cases (e.g., cortisol production) did not allow to clearly establish a tendency of evolution. Consequently, the time between assessments appeared too short to see changes for some domains of development that may require more time to change (e.g., height growth, and cognitive development).

Thirdly, some instruments in use were not validated to the Portuguese population (e.g., Bayley Scales of Infant and Toddler Development, Third Edition, 2006). Thus, cultural differences may not be accounted for when we use, as the reference values, the USA population normative values. Another limitation is that salivary cortisol was sampled during one day at each moment of assessment (T0, T1, T2, and T3), rather than on several days to incorporate the possible instability of the cortisol values. In this respect, the absence of a control sample (e.g., community sample) and/or normative values for cortisol levels made it impossible to draw further conclusions from our results. Also, the fact that caregiver's sensitivity was assessed from only 20 minutes of play rather than from more naturalistic interaction may have constituted a limitation to the coding process and contributed to the absence of the expected associations.

Finally, some results could be explained in some cases by genetic mediation, which we were not able to account for. Further studies would benefit from assessing both children and biological parents. Indeed, in some cases, programming effects may be confounded with genetic influences (O'Connor et al., 2003).

Future research should invest in increasing the sample size in order to be able to perform more complex and robust statistical analysis, identifying developmental trajectories and their predictors. Upcoming studies should make an effort to follow the development of children for longer in order to track changes that need more time to emerge. The constitution of a control sample would be of utmost importance for the interpretation of cortisol results since there are no normative values to make comparisons and infer from the clinical relevance of the cortisol levels. Other studies must also focus on obtaining more consistent information about the biological family and previous child rearing conditions (pre and postnatal), including, when possible, biological and genetic data enabling studies of genetic transmission.

In summary it is important to emphasize here the exploratory nature of this study, which due to its limitations does not allow more conclusions to be drawn, but raises new questions and contributes to a better understanding of the reality of children living in institutions in Portugal.

4. PRACTICE, SOCIAL and POLICY IMPLICATIONS

The findings from the present study revealed that although deprivation in Portuguese institutions is not so severe as in other countries, especially in Easter Europe, child development is compromised while children are being reared under institutional environments. Studies comparing children reared in foster care and from institutions have been pointing to the first as a preferential solution when adoption is not possible (Nelson, et al., 2007; van den Dries, et al., 2010). Placing children in foster families is unusual in Portugal, and it implies that a shift is made to create a system of alternative family care instead of institutional care (McCall, 2011). However, while placement in foster care and adoption are not the primary solutions and there are still children living in institutions, we should seek to improve rearing conditions in institutions. As our study revealed, some characteristics of the institutional environment contributed to better developmental outcomes (e.g., developmental activities in the institution promoted motor development) and interventions should seek to modify some features of the institutional structure and organization.

Taking into account the results from our present study, other studies that intervened successfully in the institutional context (The St. Petersburg – USA Orphanage Research Team 2008; Sparling, Dragomir, Ramey, & Florescu 2005) and contributors to the field (McCall, 2011), it is of paramount importance to develop interventional plans aimed both at improving the institutional care at various levels of action and at creating a child welfare system based on family care alternatives.

In order to achieve this, a first step has to be given: recognition that a problem exists (McCall, 2011). In the opinion of the author, and consistent with our view about Portuguese institutions, institutional caregivers and technical teams working at the institutions are well-intended people, that recognize that a family would be better for children, but they are not totally aware of the long-term consequences of traditional institutional rearing (McCall, 2011). Also, policy makers, medical professionals, social workers and general community are not aware that this welfare response, in general, failed to improve and poses serious risk to child's developmental outcomes. A primary work of alertness and awareness should be done, although caution should be taken in order to not develop or increase a stigma surrounding institutionalized children (McCall, 2011). In addition to the developmental costs that institutionalization brings, it is worth

to alert to the socio-economic costs in the short and long term resulting from the maintenance of children in institutions. Indeed, adults that spend part of their childhood in institutions tended to have more health problems, showed more marital problems, and also other kind of social and emotional behavioral difficulties which represent a cost to society, such as adolescence pregnancy, drug and alcohol abuse-related problems and criminality (Julian, 2009 in McCall, 2011). It is also cheaper to governments to sustain a family-rooted child welfare system that includes supporting biological families in order to avoid institutionalization, support foster care and adoption. However, a considerable initial investment is necessary to create such a system and maintain the quality of a working professional infrastructure, along with other difficulties that are prominent (McCall, 2011).

Creating a an alternative family-based welfare system

A first difficulty which seems transversal to countries, including ours, is the recognition by those responsible for the current system of institutional care, that this failed to provide adequate care for child's normative development (McCall, 2011). In countries where there is no or scarce tradition in fostering children, as is our case at least regarding foster families, it might be especially difficult to recruit enough foster and adoptive families to support an alternative system of family care (McCall, 2011). Transition of children from institutions to families imply that institutions lose a considerable amount of their financial support, as they are paid per capita fees. Thus, it is essential that there are modifications in the institutional financial systems. In this sense institutions should be proposed to act as partners in this family-based system, providing their facilities and their staff to be foster parents or to work together in assisting foster families (McCall, 2011). Another pertinent problem in our country, is that children stay in institutions for long times in most cases, with no opportunity to transit to a family context (e.g., adoption) due to the policy that allow birth parents to retain legal custody of children even without visiting them. Accordingly, it urges that a balance is achieved that considers parental rights but does not neglect the child's rights to a better life (McCall, 2011). The existent infrastructures of social services are not able to support this family care system in most of the countries, including ours. An effort is needed in creating and maintaining a social service to select foster or adoptive

parents, train and support them when they face difficulties. This implies also continual training, mentoring and supervision for these professionals, which take time to develop (McCall, 2011).

While the above proposals are not developed and implemented, children who remain institutionalized should not be forgotten and efforts should be made to improve their rearing conditions, through some changes proposed below based on the results of our study and others (The St. Petersburg – USA Orphanage Research Team 2008; Sparling et al., 2005).

Improving institutional care

a) *Training and supervision of the educational and technical team* with a training plan that addresses issues such as *identifying the main tasks of child development in each age; activities that promote development for each age; strategies to stimulate mental development implemented on daily routines; the importance of free play for child's development; the importance of caregiver's sensitivity and cooperation when interacting with the child; learning child's signals and how to respond to them in a responsive way; the importance of avoiding atypical behaviors when interacting with the child; the importance of individualized care and attachment.* Alongside this training period, it should be developed a continuous supervision of the educational and technical team, promoting opportunities for reflection and discussion within the working team. Professionals must do daily records of their observations and difficulties in implementing the training contents, to be discussed at these meetings. Special attention should be given to younger children since they revealed to be more fragile to the institutionalization effects.

b) *Structural changes* that include strategies as reducing the number of children in each group, forming small groups of children and caregivers, and if possible a few groups within each institution with their own spaces, making the environment of each group closer to that of a family; assignment of a primary caregiver to each child at the

admission and throughout time at the institution; keeping the child in the same group throughout their stay in the institution, being cared for by the same group of caregivers across time; arrangement of the routines so that they represent privileged opportunities for stimulation and child-caregiver interactions. These strategies aim to reduce turnover among caregivers and to increase individualization of care, in order to create opportunities for attachment relationships to emerge.

c) *Create a protocol of child's integration in the institution,* since this is a highly stressful moment for the child, and he/she need to be supported in his/her adaptation to a new environment, people and routines. Child's first days at the institution should be monitored in order to detect early difficulties and problems and consequently promote strategies for child integration.

Interventions outlined above will be more successful if implemented together. Training staff only is not sufficient and the results are better if the training is accompanied by structural changes (The St. Petersburg – USA Orphanage Research Team 2008).

PHYSICAL GROWTH, MENTAL DEVELOPMENT AND NEURO-ENDOCRINE FUNCTIONING IN PORTUGUESE INSTITUTIONALIZED CHILDREN: A LONGITUDINAL STUDY.

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