THE EFFECT OF CHRONIC PSYCHOSOCIAL STRESS ON CHILDREN'S HEALTH BEHAVIOUR AND BODY FATNESS.

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The effect of chronic psychosocial stress on children's health behaviour and body fatness.

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Believing in yourself

is the first secret to success.

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ABBREVIATIONS

%E	percent of total energy intake
ACTH	adrenocorticotropic hormone
ADP	air-displacement plethysmography
AFA	arm fat area
AINC	absolute increase of cortisol
ANS	autonomic nervous system
AR	autoregression
AUCg	area under the curve with respect to the ground
BIA	bioelectrical impedance analysis
BMI	body mass index
CAR	cortisol awakening response
CASE	child and adolescent self-harm in Europe
CFI	comparative fit index
ChiBS	children's body composition and stress
CHS	children's daily hassles scale
CLES-C	Coddington life events scale for children
ConI	conicity index
CRH	corticotrophin releasing hormone
CSS	composite stress score
CUS	children's daily uplifts scale
CV	coefficients of variation
DEBQ	Dutch eating behaviour questionnaire
DEXA	dual-energy X-ray absorptiometry
DSM	diagnostic and statistical manual of mental disorders
Fat%	fat percentage
FFM	fat-free mass
FFQ	food frequency questionnaire
FFT	fast Fourier transformation
FM	fat mass
HF	high frequency
HLM	hierarchical linear modelling
HPA	hypothalamus-pituitary-adrenal
HRV	heart rate variability
ICC	intraclass correlation
ICD	international classification of disease
IDEFICS	identification and prevention of dietary- and lifestyle-induced health effects in children and infants
IOTF	international obesity task force
ISCED	international standard classification of education
LF	low frequency
LOA	limits of agreement

LPL	lipoprotein lipase enzyme
MRI	magnetic resonance imaging
mRR	mean RRI
MUAC	mid-upper arm circumference
MVPA	moderate-to-vigorous activity
NPY	neuropeptide Y
nu	normalized units
PA	parasympathetic activity
pNN50	percentage of consecutive normal RRI differing more than 50 ms
REM	rapid eye movement
RI	Rohrer's index
RMSEA	root mean square error of approximation
RMSSD	root mean square of successive differences
RRI	RR-intervals
SA	sympathetic activity
S_b	square root of estimated between-person variance
SD	standard deviation
SDNN	standard deviation of the normal RRI
SDQ	strengths and difficulties questionnaire
SEE	standard error of the estimate
SEM	structural equation modelling
SSF	subscapular skinfold
S_w	square root of estimated within-person variance
SWS	slow wave sleep
TSF	triceps skinfold
VIGeZ	Flemish institute for health promotion
VO_2max	maximal oxygen uptake
WASO	wake after sleep onset
WHO	world health organization
WHR	waist-to-hip ratio
WHtR	waist-to-height ratio

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INTRODUCTION

1. Stress

1.1. Definition of stress

Several definitions for stress exist. The most used definition is based on the transactional theory (**interaction person/environment**): "Stress involves a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well being" (Lazarus and Folkman 1984). Consequently, stress arises when the demands of a situation exceed an individual's ability to cope and resolve the problematic situation, resulting in emotional, behavioural and cognitive disturbances (McCance, Forshee et al. 2006).

Stress is an adaptive, dynamic state. Consequently, the concept stress is composed of several aspects as shown in Figure 1. The initiating stimulus is the **'stressor**'. This is the environmental demand, challenge or event. When being confronted with these stressors, people evaluate whether this is a potential threat. This is the **stress appraisal or perceiving phase**. When homeostasis is threatened i.e. when there is a discrepancy between what is expected or the 'normal' situation (set point) and what is happening in reality (actual value), a **physiological and psychological coping** response will be initiated that induces arousal. It reflects the expectancy that he or she will be able to handle the situation and react to it with a positive result. If this result is not positive, the coping response can trigger **emotional, behavioural and cognitive disturbances** that might put a person at risk for **psychiatric and physical illness** when these disturbances are intense (Ursin and Eriksen 2004).

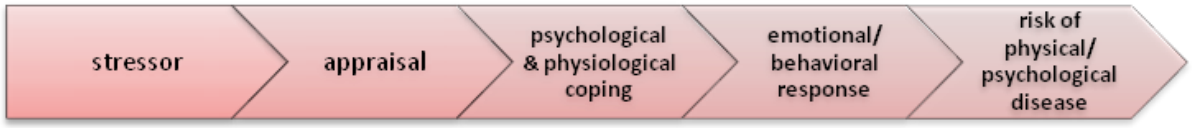


Figure 1: Simplified model of the stress process.

Nevertheless, not all stressors will trigger emotional and/or behavioural disturbances and illness. After all, the stress response is a positive alarm by mobilizing physiological resources to initiate and improve performance. As a result, the body will try to regain the balance or homeostasis. Figure 2 summarizes the essential components for pathological stress. The likelihood for a negative outcome is highest when the demands are high and the coping or

control is low. **Chronic, unpredictable and uncontrollable stressors** are the most difficult to cope with. These terms are subjective since it is the perceived chronicity (duration), predictability (probability to occur) and controllability (capacity to influence the situation) that matters. Unpredictable stressors will be accompanied by an absence of an anticipatory hormonal response, while uncontrollable and chronic stressors will be accompanied by high stress hormones with a reduced recovery afterwards. Since the arousal will sustain until the reason for the arousal is eliminated, these difficult situations will not lead to phasic arousal but to sustained, chronic arousal. It is this **chronic arousal or chronic stress** that can initiate pathophysiological processes of illness (Ursin and Eriksen 2004, Koolhaas, Bartolomucci et al. 2011).

The goal of the body in stress situations is to maintain stability through changes. This short-term adaptation in the face of environmental challenges is called allostasis. Three highly-integrated systems mediate this allostasis: the immune system, the nervous system and the endocrine system. In this allostasis, the stressor triggers a response for an appropriate time which then turns off again. In contrast, chronic stress leads to prolonged activation or inefficient management of these allostatic systems (=allostatic load), with detrimental physiological consequences such as cardiovascular disease, neurodegeneration and metabolic syndromes (e.g. insulin resistance and obesity) (McEwen 1998, McEwen 2007). Also adverse childhood experiences have been shown to influence the responsiveness of the allostatic systems (which are still maturating during childhood) and to induce allostatic load with changes in the nervous, endocrine and immune systems during childhood and future adulthood (Danese and McEwen 2012).

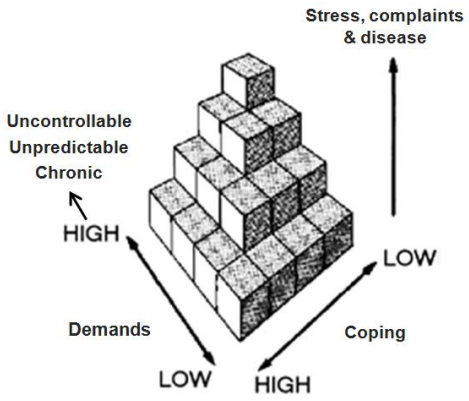


Figure 2: Concept of stress (Ursin and Eriksen 2004).

1.2. Stressors

Based on the definition of stress, stressors are defined as internal and/or external demands that are appraised as taxing or exceeding the resources of the person. Stressors can be **physiological** (e.g. cold and pain) or **psychosocial** (psychic and social aspects). For modern mankind, psychosocial stressors are the most common. Besides life events, which are assumed to profoundly affect someone's life, daily annoyances and minor hassles are also seen as important stressors (Kanner, Coyne et al. 1981). Also in childhood, plenty of stressors exist (Table 1). Of course, the **nature of stressors evolves over centuries** due to socio-political changes. In 1970-1990, children mainly reported on family problems (e.g. angry parents), losing people (e.g. by death), psychological (incompetence feelings) and behavioural (getting in trouble) problems while from 1990 on extra stressors concerned social problems (e.g. bullied), safety (violence, abuse, accidents) and 'no time to just play' (due to school or hobbies) (Ryan-Wenger, Sharrer et al. 2005). In today's society, parents may feel pressure to offer their children a lot of opportunities hereby enhancing their child's potential: children may be in sports, lessons, camps or competitions year round. This increases the feeling to compete with their peers, while the demands of coaches and parents may be an unrealistic expectation according to the developmental level of their child.

Table 1: Stressors during childhood (Ryan-Wenger, Sharrer et al. 2005).

1970-1990	New since 1990 or 2000
Parents mad or yells at me	Problems with sibling
Parents divorced or fighting	Being made fun of or bullied
Being poor	Being alone
Moving	Getting hurt (by accident)
Death of family member or friend	Violence
Sick, dying or lost pet	Abuse
Losing a friend	Tests
Bad grades	Too much homework
Pressure from parents or friends	Too many things to do
Getting in trouble	
Feeling bad about myself (performance, appearance)	
Feeling sick or not feeling good	

Also in European children, stressors are **highly prevalent**. In the European IDEFICS sample of 4-11y old children, 53.4% lived in familial/social adversities and 40.3% experienced at least one major negative life event such as a parental divorce (Vanaelst, Huybrechts et al. 2012). More specifically, between 6-58% (average 23%) of the 11y olds in the international HBSC study reported to be pressured by schoolwork and between 2-23% (average 12%) reported to be bullied at school (Currie, Zanotti et al. 2009). For both stressors, Flemish children scored around the average.

1.3. Emotional and behavioural problems

Mental health disorders have been catalogued in **clinical manuals** such as the 'International Classification of Disease' (ICD) organized by the World Health Organization (WHO) and the 'Diagnostic and Statistical Manual of Mental disorders' (DSM) by the American Psychiatric Association. Apart from these categorical scales to detect the clinical absence or presence of a disorder, also continuous scale parameters have been used in **public health** to detect emotional/behavioural symptoms, perceived stress or quality of life (Cohen, Kessler et al. 1997, Achenbach, Rescorla et al. 2012). Emotional and behavioural symptoms have often been categorized in **internalizing** (based on depression and anxiety) and **externalizing** (based on conduct and attention-deficit hyperactivity disorder). This is the underlying idea of many questionnaires such as the 'Strengths and Difficulties Questionnaire' (Goodman 1997).

Mental health problems or emotional/behavioural problems affect **10-20% of children and adolescents worldwide** (Brauner and Stephens 2006, Kieling, Baker-Henningham et al. 2011). In Flanders, 24% of the patients in mental health care are children and adolescents (VAZG 2011). In the international 2009/2010 HBSC study, between 70 and 96% (average 88%) of 11y olds reported high life satisfaction. Low life satisfaction might be explained by the high prevalence of psychosomatic complaints (physical complaints without a medical explanation) and psychological problems in childhood. Between 12 and 65% (average 28%) of the 11y olds in the HBSC study reported these problems more than once a week (Currie, Zanotti et al. 2009). Also in the European IDEFICS 4-11y old sample, 45.5% of children experienced at least one psychosomatic or emotional symptom, with low emotional well-being during the last week being most frequently reported (38.2%) (Vanaelst, De Vriendt et al. 2012). Frequent psychosomatic complaints are headache (prevalence 10-30%), gastro-intestinal issues (prevalence 7-25%) and fatigue (prevalence 50%) (Dell and Campo 2011).

1.4. Stress endocrinology and its measurement

As described above, the physiological stress response aims to regain homeostasis by giving priority to essential body functions. As shown in Figure 3, two endocrine systems are regarded as primary components of the stress response: the **hypothalamic-pituitary-adrenal** (HPA) axis and **autonomic nervous system** (ANS). The HPA axis is postulated to be mainly sensitive to differences in affective valence (pleasure vs. displeasure), while the ANS axis is more sensitive to differences in activation (low vs. high) (Holmes, Ekkekakis et al. 2010).

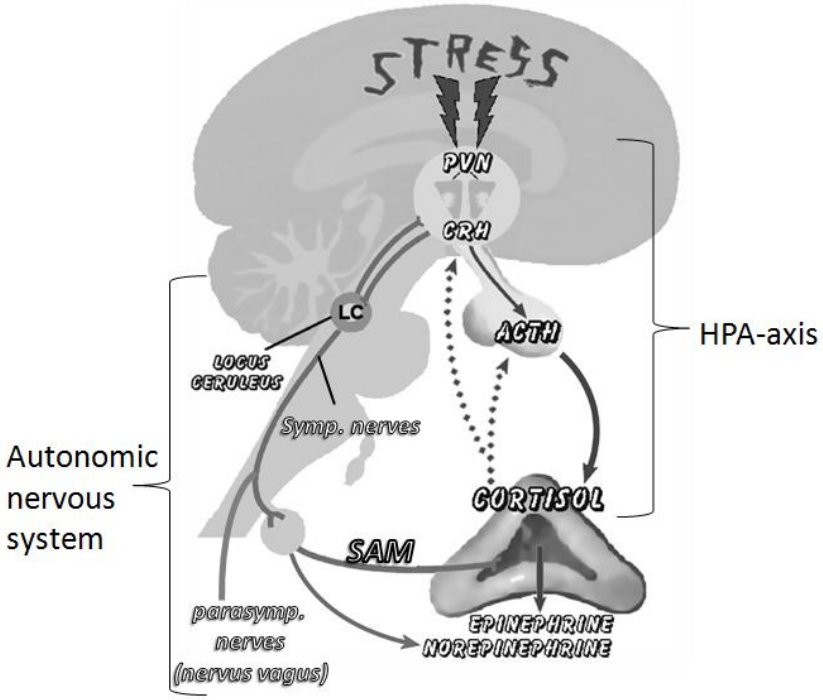


Figure 3: Physiology of the stress response (Kyrou and Tsigos 2009).
PVN= paraventricular nucleus; CRH= corticotrophin releasing hormone; ACTH=adrenocorticotropic hormone; HPA= hypothalamic-pituitary-adrenal axis; SAM=sympatho-adrenal-medullary system

1.4.1. HPA axis

The HPA reaction starts in the paraventricular nucleus of the hypothalamus with the secretion of **corticotrophin releasing hormone** (CRH) (and also arginine vasopressin) that stimulates the pituitary gland to secrete **adrenocorticotropic hormone** (ACTH). This ACTH finally leads to **cortisol** secretion in the adrenal cortex. Cortisol is the main end product of the HPA axis. Consequently, cortisol is used as a biomarker to assess the HPA activity. In the blood circulation, up to 95% of the cortisol is bound to corticosteroid-binding globulin and albumin, the other 5% of free cortisol is the biological active fraction. This free cortisol binds to two types of intracellular receptors, the glucocorticoid receptor and the mineralocorticoid receptor.

The secreted cortisol also initiates a **negative feedback** loop to the hypothalamus and the pituitary to limit the duration of the HPA stress response (Tsigos and Chrousos 2002).

Apart from blood measurements, cortisol is now also routinely measured in **saliva**. Since unbound cortisol is small and lipid-soluble, it will passively diffuse from the blood through the cell-membrane of the salivary gland cells. Consequently, the salivary cortisol concentration is a reliable reflection of serum free cortisol that is biological active. Due to its non-invasive and pain-free character, salivary cortisol sampling allows sampling several times a day in the natural environment (Tornhage 2009).

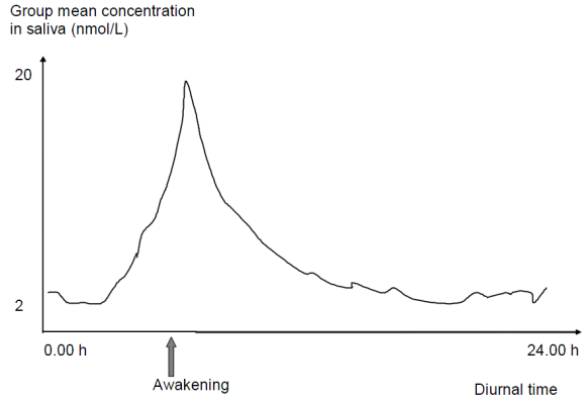


Figure 4: Circadian rhythm of salivary cortisol (Kristenson, Garvin et al. 2012).

Cortisol secretion (both in blood and saliva) has a **circadian rhythm** with lowest levels in the first half of the night, a peak in the early morning and a diurnal decline (see Figure 4). Apart from this circadian rhythm, there is also a **cortisol awakening response** (CAR) showing a quick cortisol increase within 30 minutes after wake up (Fries, Dettenborn et al. 2009). This circadian rhythm is caused by the connection with the suprachiasmatic nucleus, our internal clock which receives light information from the retina. Moreover, this circadian rhythm is actually made up from changes of a much faster underlying **ultradian rhythm** with pulses that occur approximately one hourly (Dickmeis 2009).

Stress has often been associated with higher cortisol values (Selye 1956, Michaud, Matheson et al. 2008). Nevertheless, the associations are quite complex, giving conflicting results and a **hyper-/hypocortisolism hypothesis** was published suggesting that recent exposure to a stressor may initially elevate cortisol levels, while the HPA axis may develop a counter-regulatory response of cortisol lowering after extended exposure to stress (Heim, Ehlert et al. 2000). This hypocortisolism is an allostatic situation characterized by lower morning cortisol,

higher evening cortisol and a flatter diurnal slope (see Figure 5). Studies in adults have suggested a steeper slope as a more dynamic and probably healthier response (Kristenson, Garvin et al. 2012). The question remains whether this statement is also applicable in children. Although some studies found this hypocortisolism pattern in children (Gunnar and Vazquez 2001), hypocortisolism may be less frequent in children as some time is needed before the hypercortisolism counter-regulatory response is induced. A recent study showed that adversities during childhood were associated with a high cortisol level, while adversities during adolescence were associated with a low cortisol level (Bosch, Riese et al. 2012). Moreover, it is worth to notice that early life stressors may have a permanent effect on the stress reactivity in later life. Adolescents with perinatal adversities showed an excessive cortisol reactivity (Bosch, Riese et al. 2012).

Apart from this, variability in cortisol response is attributable to the **nature of the stressor** (social or physical) and also to **the person facing it** (emotional response and psychiatric sequelae) (Miller, Chen et al. 2007). To attribute salivary cortisol correctly as a stress biomarker, the biological and methodological variation need to be considered (Hansen, Garde et al. 2008, Adam and Kumari 2009, Kudielka and Wust 2010), also in children (Hanrahan, McCarthy et al. 2006, Jessop and Turner-Cobb 2008).

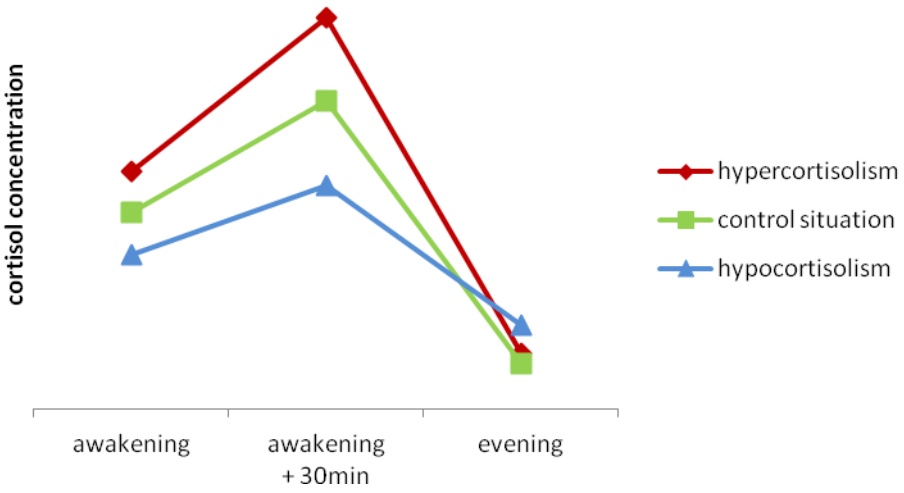


Figure 5: Hypothetical cortisol pattern explaining the concepts of hyper- and hypocortisolism.

1.4.2. Autonomic nervous system

As shown in Figure 3, the catecholamines **adrenaline and noradrenaline** are the main hormonal end products of the autonomic nervous system. The system starts in the brainstem from where the signal is transmitted by nerves through the spinal cord to reach peripheral organs. These autonomic innervations can be divided in the **sympathetic system** that prepares the body for a fight or flight in times of stress and the **parasympathetic system** that brings the body back from an emergency status to a resting status. In times of stress, this autonomic system will give priority to cardiovascular tone and high blood pressure, respiration and release of energy substrates, while it will temporarily suppress digestion, growth, reproduction and immunity (Charmandari, Tsigos et al. 2005). In the locus coerulus (located in the brainstem) an important branching of the sympathetic system innervates the **adrenal medulla** which secretes adrenaline and partially also noradrenaline. This system is called the sympathoadrenal system.

An increasingly used quantitative marker of the ANS activity is the **heart rate variability** (HRV). HRV is defined as the variability of the distance between consecutive R peaks of the electrical heart beat signal (see Figure 6) (Task Force 1996). The sympathetic and parasympathetic innervations (mainly the nervus vagus) on the sinus node of the heart control the firing of electrical impulses that stimulate heart contraction. The sinus node fires at an intrinsic rate, but the parasympathetic activity regularly sends inhibitory signals with a temporarily reduction of the heart rate. These changes reflect the heart's ability to respond to physiological and environmental stimuli. Subsequently, a reduction of HRV (i.e. reduced parasympathetic with or without increased sympathetic activity) is associated with increased morbidity and mortality (Thayer, Yamamoto et al. 2010). Consequently, HRV may be a potential pathway linking stress to ill health (Thayer and Brosschot 2005). In children, the longitudinal relationship of HRV with cardiovascular diseases has not yet been examined, but children's HRV (lower parasympathetic activity and a changed balance) has been associated with cardiovascular risk factors such as systolic blood pressure and obesity (Zhou, Xie et al. 2012).

Figure 6: The electrical heart beat signal and analysis of heart rate variability.

The use of HRV as a stress marker is common in adults, but the **application in children is still scarce**. Low HRV has previously been linked with poor emotion regulation, high stress, anxiety, decreased stress reactivity and increased stress vulnerability (Porges, Doussard-Roosevelt et al. 1994, Porges 1995, Friedman 2007). Apart from HRV, also other parameters for this stress axis have been used such as the salivary alpha-amylase concentrations (Granger, Kivlighan et al. 2007).

1.4.3. Interactions between systems

There are multiple sites of interactions between the HPA and ANS axes. CRH and noradrenaline stimulate each other through **CRH and noradrenergic receptors**, but at the same time auto regulatory negative feedback loops exist. Moreover, cortisol itself can also influence the noradrenergic system.

Both pathways also interact with three **higher brain control areas**

(1) that influence affect and anticipatory phenomena (mesocorticolimbic reward systems);
(2) the initiation, propagation and termination of stress system activity (amygdala/hippocampus complex);
(3) the setting of the pain sensation (the hypothalamic arcuate nucleus) (Tsigos and Chrousos 2002, Charmandari, Tsigos et al. 2005).

Both pathways also interact with thermoregulatory and appetite-satiety centres of the central nervous system, as well as the growth, thyroid, reproductive and immune axes (Charmandari, Tsigos et al. 2005). As a result, chronic activation of these two systems will inevitably have a wide range of health consequences.

1.5. Medical and financial consequences of stress

As was explained in Figure 2, mainly chronic, uncontrollable and unpredictable stressors will initiate pathophysiological pathways. Stress is not only linked to **psychological** but also to **physiological** health complaints. After all, the stress system will give priority to certain bodily functions over other functions such as growth, digestion, immune function and reproduction. Moreover, cortisol as a glucocorticoid modulates the expression of approximately 10% of the human genes (Buckingham 2006) amongst others those involved in inflammation and metabolism. Also the catecholamines exert regulatory effects on several systems such as the cardiovascular, pulmonary, hepatic, skeletal muscle and immune system. Accordingly, cancer,

the progression of aids, inflammatory, pulmonary, gastrointestinal and cardiovascular diseases have been associated with stress (Cohen, Janicki-Deverts et al. 2007). The current thesis will examine whether there also exists a longitudinal relation between stress and the prevalence of obesity.

Mental health is the leading cause of **health-related disability**, accounting for 15-30% of the disability-adjusted life-years lost during the first three decades of life (Lopez and Disease Control Priorities Project 2006). This brings along a **high cost for society**. First of all, medical costs are high (Lynch and Clarke 2006) since negative events and psychosocial problems in childhood have been related to adult psychiatry (Fryers and Brugha 2013). Apart from these medical costs, also increased costs for the society due to crime, substance use, unemployment, lower salary (approximately 20% lower) and personal relationship problems have been reported (Scott, Knapp et al. 2001, Fergusson, John Horwood et al. 2005, Smith and Smith 2010).

2. Obesity

2.1. Epidemiology of obesity

Obesity is defined as an **excess of body fat** (WHO 2000). The prevalence of obesity is determined based on the **body mass index** (BMI) as kg/m². In children and adolescents, anthropometric measures and body composition change considerably over the years as they grow. Body composition and body mass distributions are **sex and age related** in both children and adolescents. Therefore, growth charts and population references are necessary to define obesity. In 2000, the International Obesity Task Force (IOTF) proposed BMI cut-off points for screening which correspond to BMI values of 25 and 30 kg/m² at the age of 18 years (Cole, Bellizzi et al. 2000). Other studies have used the BMI-for-age percentile derived from international or national population data represented on charts with obesity defined as a BMI higher than the 95th percentile for a specific age and sex group and overweight as a BMI between the 85th and the 95th percentile. To detect children over the 95th percentile or more than 2 standard deviations (SD) higher than the population mean, z-scores are often calculated as '(measurement-mean)/SD'.

Although BMI is the most used measurement to determine obesity due to low cost and time requirements, other anthropometric measures have been applied as well. Routine measures can give an indication of the **fat distribution** over the body: subcutaneous fat (underneath the skin) by skinfold thickness versus visceral fat (around the abdominal organs) by waist circumference. Knowledge of the fat distribution is interesting since visceral fat is the more pathogenic fat. When accurate measures of total or regional body fat percentage are required, more **sophisticated methods** can be used such as air-displacement plethysmography (ADP), dual-energy X-ray absorptiometry (DEXA) and magnetic resonance imaging (MRI) (Sweeting 2007).

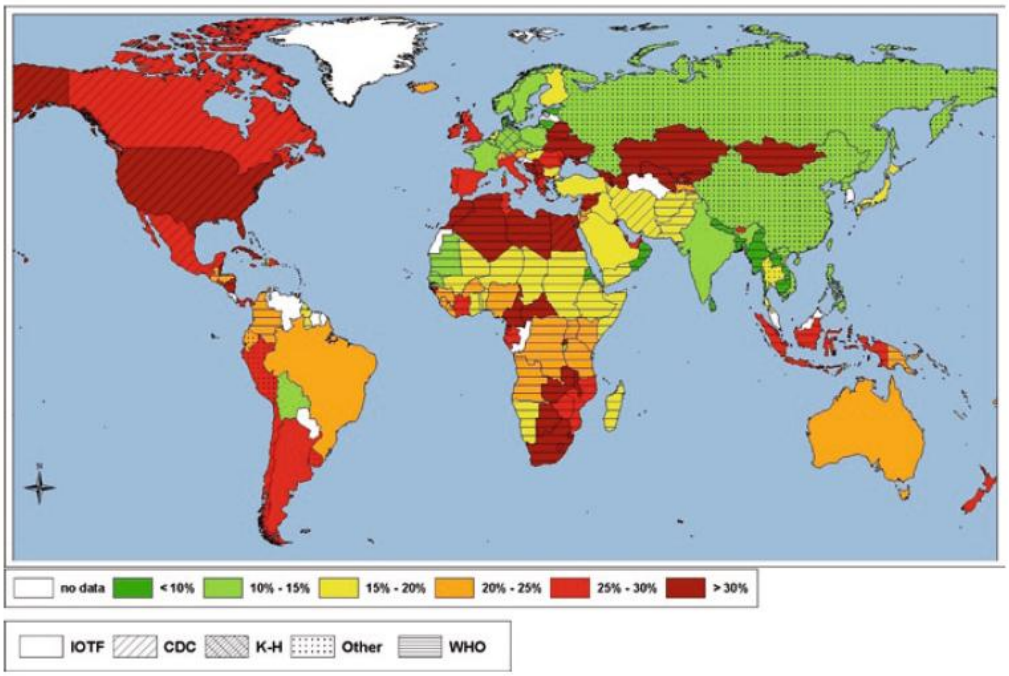


Figure 7: Worldwide prevalence of overweight and obesity in children and adolescents (Moreno, Pigeot et al. 2011).

The last decades have been characterized by a **global growing obesity epidemic**. In the European health interview survey of 2008/2009, the proportion of overweight/obese people varied between 27 and 70% with numbers between 8 and 24% for obesity (Eurostat 2011). More alarming is the increase in **childhood obesity** with world-wide at least 110 million overweight/obese children (Moreno, Pigeot et al. 2011). Figure 7 shows the numbers for worldwide overweight/obesity prevalence in children and adolescents. Highest values were seen in the US, the south of South America, in some Middle East and northern African countries like Egypt, in southern Africa, central Asia, Indonesia and New Zealand. In the European Union, the prevalence of childhood overweight and obesity ranges from 10-20% in northern European areas to 20-40% in Mediterranean Sea countries, the UK and some Eastern countries (Moreno, Pigeot et al. 2011). In Belgium, the rates are not as high as in some other European countries but rates have been tripled in two decades and a prevalence of 17% in 2-18y olds and even 22% in 5-9y old has been reported in the Belgium national health survey of 2008 (WIV 2008). In most countries, the obesity epidemic is still growing with the exception of the US, Australia and some European countries where the trend seems to have reached a plateau in recent years (Moreno, Pigeot et al. 2011). Nevertheless, the high prevalence numbers stress the importance of a better understanding of the complex obesity aetiology to help developing effective prevention programs.

2.2. Medical and financial consequences of obesity

Figure 8 summarizes the medical complications associated with obesity. The most frequent obesity-related problems in children and adolescents include **psychosocial problems**, increased **cardio-vascular** risk factors (dyslipidaemia, hypertension and abnormal glucose metabolism) and **hepatic-gastrointestinal** disturbances. Nevertheless, the most important long-term consequence of childhood obesity is its **persistence into adulthood** ("tracking phenomenon") (Singh, Mulder et al. 2008) since 80% of the obese 9-year olds stay obese for the rest of their life (Whitaker, Wright et al. 1997). Moreover, they experience increased **metabolic complications** in adulthood with up to 7.5% of explained mortality rates today (Reilly and Kelly 2011). International studies on the **economic costs** of obesity have shown that they account for 2% to 7% of total health care costs in developed countries (WHO 2000).

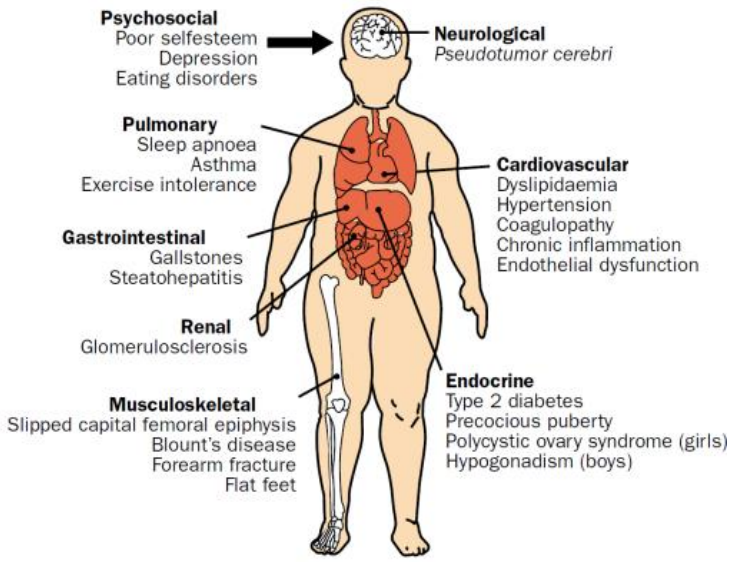


Figure 8: Medical complications associated with obesity (Ebbeling, Pawlak et al. 2002).

2.3. Mechanisms and prevention of obesity

The development of childhood overweight involves a complex set of factors from multiple contexts that interact with each other to place a child at risk. Figure 9 depicts the **ecological model** that has been developed (Davison and Birch 2001, Skelton, Irby et al. 2011): a composite set of factors from multiple contexts at the individual and environmental level and interactions between both levels. Beginning at the level of the child, lifestyle factors can place a child at risk for the development of overweight. **Four lifestyle factors** that have been

associated with stress are an unhealthy diet, low physical activity, high sedentary behaviour and poor sleep. The impact of child risk factors on the development of overweight is moderated by child characteristics such as age, sex and genetics. These factors do not have to be considered in isolation, rather they work together to determine overweight development. In children, these individual characteristics are surrounded by ecological niche factors that include the family and the school, which are in turn embedded in larger social contexts including the community and society at large.

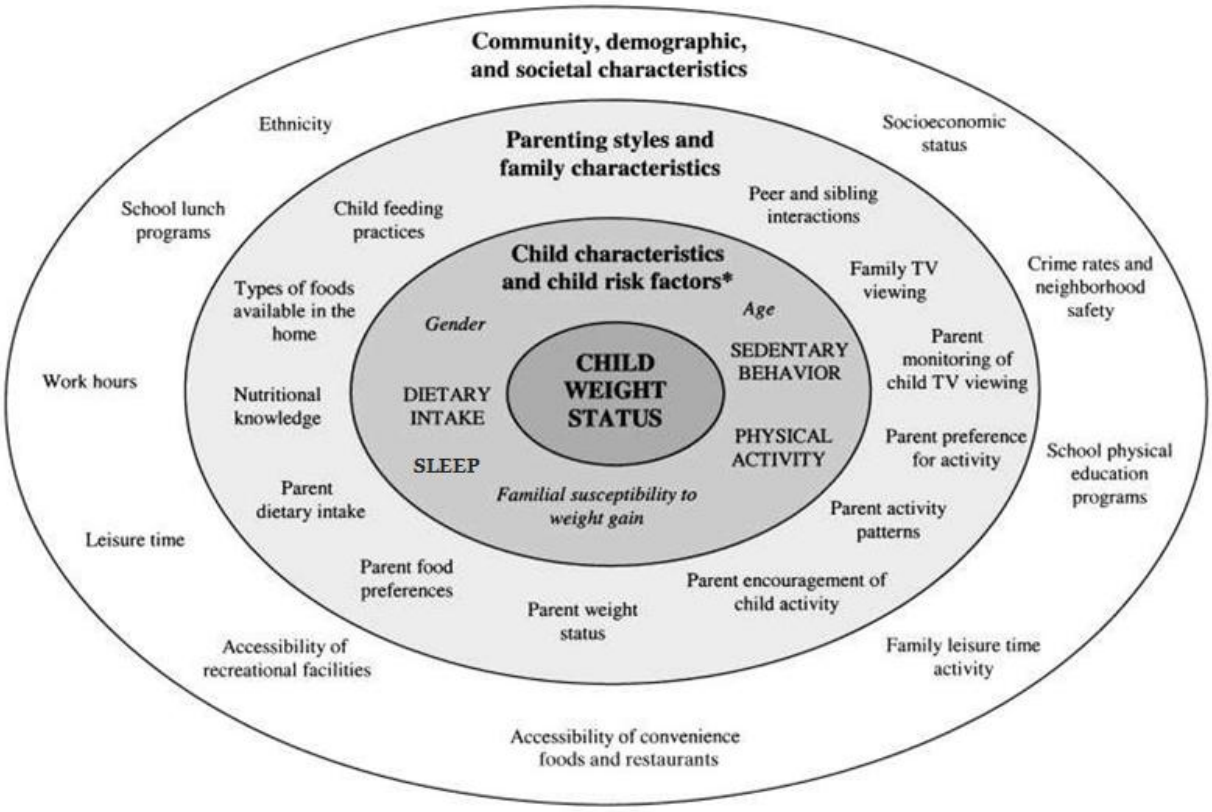


Figure 9: Ecological model of predictors of childhood overweight (Davison and Birch 2001).

A Cochrane meta-analysis on 55 prevention studies in children and adolescents concluded that there is evidence to support beneficial effects of **child obesity prevention** programs on BMI, particularly for children between 6 and 12y. However, there was a high level of observed heterogeneity and a likelihood of small study bias (Waters, de Silva-Sanigorski et al. 2011). Promising preventive actions included 1) a school curriculum that covers healthy eating, physical activity and body image; 2) increased sessions for physical activity and the development of fundamental movement skills throughout the school week; 3) improvements in nutritional quality of the food supply in schools; 4) environments and cultural practices that support children to eat healthier foods and to be active throughout each day; 5) support for

teachers and other staff to implement health promotion strategies and activities; and 6) parent support and home activities that encourage children to be more active, eat more nutritious foods and spend less time in screen based activities.

Recently, the WHO statement "**No health without mental health**" (WHO 2005) has emphasized the importance of mental well-being. Also for the aetiology of obesity, psychological dimensions have received increasing interest (Karasu 2012). Putting into practice, the idea of a **biopsychosocial model** (in which not only biological but also psychological and social factors influence disease) has been hypothesized in the depression-obesity relation (Wildes, Marcus et al. 2006, Maxwell and Cole 2009). With this idea in mind, we will explore the possibility and mechanisms of a stress-obesity relation.

3. Relation stress-obesity

3.1. Short literature overview

In examining literature, the presence of a publication bias (i.e. mostly only studies with significant results are published) should be considered. Consequently, the discussed information is probably an overestimation of the reality. Moreover, stress will be considered in a broad definition including correlates such as depression.

In **adults**, bidirectional relations have been suggested (Markowitz, Friedman et al. 2008, Foss and Dyrstad 2011): stress can increase adiposity through cortisol and lifestyle, while adiposity can increase stress mainly through lower self-esteem and stigma. In a recent meta-analyses of longitudinal studies, depressed people had 58% more risk to become obese and obese people also had 55% more risk to become depressed (Luppino, de Wit et al. 2010). In another meta-analysis, work and life stress also significantly increased BMI and/or waist longitudinally (Wardle, Chida et al. 2011).

In **children**, no recent meta-analyses were found, although two recent reviews have shown an overview of the stress effects on obesity.
The first review only included longitudinal studies (Incledon, Wake et al. 2011). A summary of the results is shown in Table 2. The selected 12 longitudinal studies mainly used BMI or waist as a measure of anthropometry but one study used the more advanced DEXA technique to examine fat percentage. Concerning the predictor, studies on clinical depression, perceived

stress, anger/anxiety and behaviour were found. For most studies, no associations with body composition were found. When only considering studies with measured and no self-reported body composition, three studies found evidence for stress parameters that increased overweight: one showed an effect of clinical depression on BMI, another showed an effect of anger on waist-to-hip ratio and a last study found an effect of maladaptive behaviour on BMI. This review concluded that the evidence is low for this relation in children/adolescents. Especially **in young children more research is needed.**

Table 2: Results of a review on longitudinal stress effects on obesity in children/adolescents (Incledon, Wake et al. 2011).

	author, country	N	age	follow-up	outcome	significant findings
clinical depression	Sweeting 2005 (UK)	2127	11	4	BMI	/
	Chen 2009 (USA)	543	10	4	BMI	/
	Tanofsky-Kraff 2006 (USA)	146	6-12	4	fat%	/
	Jansen 2008 (Netherlands)	787	9-10	3	BMI	/ (positive for weight perception)
	Rhew 2008 (USA)	446	11-12	1	BMI	positive for reported BMI only
	Goodman 2002 (USA)	9374	12-18	1	BMI (report)	positive relation
	Stice 2005 (USA)	496	11-15	4	BMI	positive relation
perceived stress	van Jaarsveld 2009 (UK)	4065	11-12	5	BMI & waist	/
anger/axiety	Midei 2009 (USA)	213	14-16	3	waist/hip	positive relation for anger not for anxiety
behaviour	Lumeng 2003 (USA)	755	8-11	2	BMI	positive relation
	Mamun 2005 (Australia)	2934	5	9	BMI	/
	Bradley 2008 (USA)	1254	2-12	1-10	BMI	/

Another review focused on the effect of household and individual stressors on overweight parameters (Gundersen, Mahatmya et al. 2011). In each of the included studies, there was at least some evidence of the relationship between stressors and obesity. The used obesity parameter was almost exclusively BMI (sometimes parentally reported) and not all studies were longitudinal. Concerning the household stressors, self-esteem, financial strain, maternal depression, maternal distress and neglect showed significant associations with obesity. For the individual stressors, depression was often examined and significant, although also more broad parameters such as future life goals, substance use and overall stress perceptions were significantly related to BMI.

Apart from the stress effects on obesity, obesity effects on stress also have been investigated in children (Wardle and Cooke 2005, Griffiths, Parsons et al. 2010). Moderate levels of body dissatisfaction and a significant reduction in global self-esteem, social functioning and quality of life in obese youth was seen, but only few are depressed.

A recent UK report stresses the importance of the **bidirectional associations** between mental health and obesity. They reported that the evidence is relatively strong in teenagers and adults, but that the strength and the amount of evidence in young children is much weaker (Gatineau and Dent 2011). Main reported mediators in the effect on obesity were unhealthy lifestyle (such as food and exercise), medication side effects and reduced social support. Reported moderators were mainly age and sex.

3.2. Mechanisms of the stress effects on obesity

Several mechanisms in the effect of stress on obesity have been hypothesized as shown in Figure 10. Firstly, **direct metabolic changes** (such as increased visceral fat disposition and a stimulation of appetite) are mainly caused by a dysregulation of the stress system and the production of stress hormones (mainly cortisol). This is in agreement with the concept of stress-induced allostatic load leading to detrimental physiological consequences due to overactive endocrine reactions (McEwen 1998). Secondly, stress may indirectly facilitate obesity through **behavioural pathways** such as maladaptive coping behaviours leading to an obesity stimulating lifestyle: emotional eating of 'comfort' food (rich in sugar and fat), a disordered sleep and a lack of exercise with an increase in screen time. After all, recent research has shown **anatomical links** in the regulation of stress, energy and sleep (Rolls, Schaich Borg et al. 2010). Anatomical and functional intercorrelations exist between:

1) stress regulation in the paraventricular nucleus of the hypothalamus,

2) hypocretin cells that regulate sleep in the lateral area of the hypothalamus and

3) metabolism regulation in the arcuate nucleus of the hypothalamus.

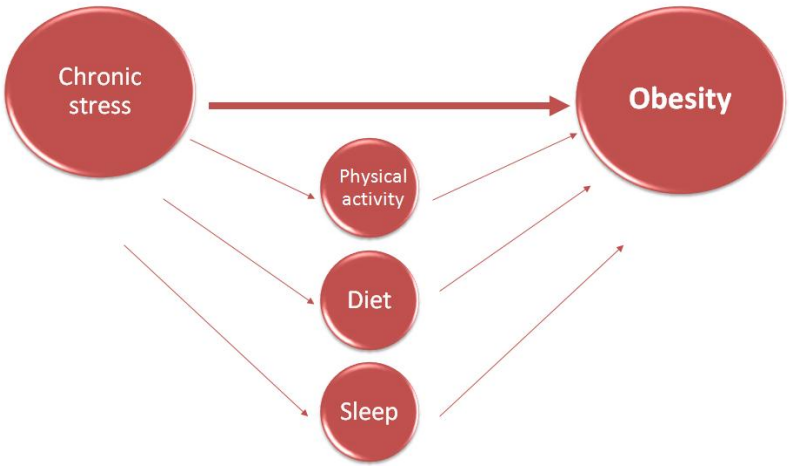


Figure 10: Hypothesized mechanisms in the effects of stress on obesity parameters.

3.2.1. Cortisol

Until now, the HPA axis appears to be involved in food intake and fat deposition more directly and by more complex pathways than the ANS axis (Holmes, Ekkekakis et al. 2010). Stress increases cortisol. This cortisol interacts with lipid metabolism in two ways as summarized in Figure 11 (Peckett, Wright et al. 2011). First, cortisol increases the amount of free circulating fatty acids by stimulating the **lipoprotein lipase enzyme (LPL)**. These free fatty acids can then be used to accumulate fat in fat cells. Secondly, cortisol influences this **fat storage**. Increased adiposity is caused by hyperplasy (adipogenesis) and in the presence of insulin also by hypertrophy (lipogenesis). Cortisol may also influence lipolysis (degradation of adipose tissue triglycerides to fatty acids). Both prolipolytic and antilipolytic activity has been hypothesized, but the mechanisms are still unclear and may depend on duration, dose and location of cortisol exposure. Antilipolytic activity has mainly been observed in high cortisol concentrations and in the abdominal region. The fat storage chiefly occurs in the visceral fat cells since the cortisol receptors have a high density in this region (Bjorntorp 2001).

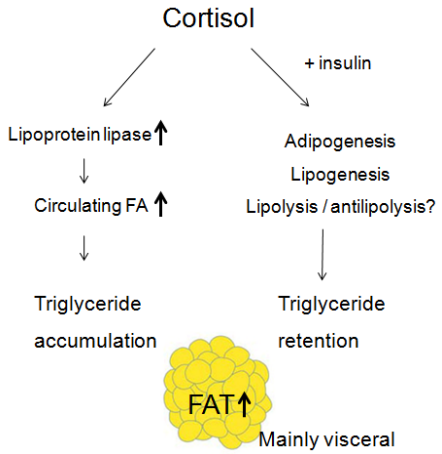


Figure 11: The effect of cortisol on adiposity.
FA= fatty acids

Mechanistic pathways have largely been investigated in animal studies, but some studies also tried to find this link in human observational studies. In identical twins (identical genetic information), a difference in visceral fat accumulation (rather than obesity in general) could be explained by higher psychosocial stress and cortisol and noradrenalin levels (Marniemi, Kronholm et al. 2002). A study in 8-11 year old girls has shown **cortisol as a moderator** in the relation between stress events and abdominal fat: a higher number of school-related negative events was related to more abdominal fat for girls with a high CAR but no such association was found for girls with a low CAR (Donoho, Weigensberg et al. 2011).

3.2.2. Diet

Stressed people may eat increased amounts of unhealthy food since eating functions as a way to cope with stress as it leads to distraction (Macht 2008). The underlying pathway is the increased cortisol during stress (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Torres and Nowson 2007, Epel, Tomiyama et al. 2012).

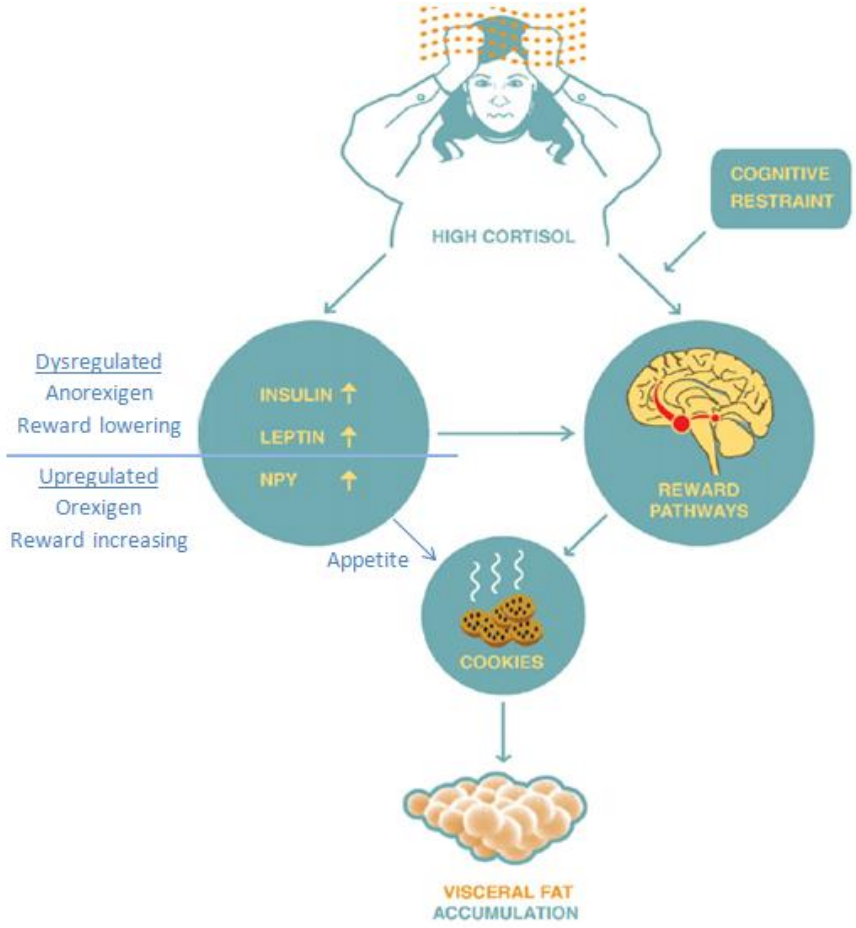


Figure 12: The effects of cortisol on food consumption (Adam and Epel 2007).

The high cortisol during stress increases **reward sensitivity and appetite** (see Figure 12). Cortisol stimulates the reward pathways (opioid and dopamine system) especially when people are on cognitive restraint. More importantly, cortisol also influences appetite hormones: it upregulates neuropeptide Y (NPY) and dysregulates insulin and leptin. The NPY increases appetite and reward. On the other hand, insulin and leptin decrease appetite and reward but due to the dysregulation the body becomes resistant and appetite and reward will be increased. Consequently, mainly rewarding food items will be consumed. These foods rich in sugar and fat have been called '**comfort food**'.

Apart from insulin, leptin and NPY as important hormones explaining the effect on appetite, recent literature also suggest an influence on other hormones like the upregulation of the orexigen ghreline (Perello and Zigman 2012, Schellekens, Finger et al. 2012).

In this regard, the association between stress and food consumption must be visible as well in people's reports on their eating behaviour. Schlundt demonstrated that different eating behaviours can be observed during a two-week period, such as external eating, restrained eating, emotional eating or binge eating (Schlundt, Taylor et al. 1991). In relation to stress, **emotional eating** is the most important. People with an emotional eating behaviour have learned to label the negative feelings of stress as 'hunger' (Bruch 1964) and will think about food as an escape from stress (Dallman, Pecoraro et al. 2003, Adam and Epel 2007). According to Van Strien, **external eating** refers to a personal trait reflected in a tendency to overeat in reaction to food cues whereas **restrained eating** means eating less than wanted when exposed to food, which is not necessarily associated with overeating (van Strien, Frijters et al. 1986). Both are however also linked with stress but the direction of the effect is less clear (Macht 2008). In the scarce literature on children and adolescents, negative emotions and problems have been associated mainly with emotional eating (Braet and Van Strien 1997, Goossens, Braet et al. 2009, Nguyen-Rodriguez, McClain et al. 2010).

In sum, the stress-diet relation is **very complex** involving multiple pathways, molecules and receptors in different brain regions. Even parallelisms of these stress-induced food cravings with addiction mechanisms have been found (Pelchat 2009, Brownell and Gold 2012). Apart from stress-induced increases of eating also stress-induced decreases of eating have been reported in the absence of palatable food or in the case of very intense emotions (acute stress) (Macht 2008, Maniam and Morris 2012). Moreover, **reversed causation** might also be present by dietary patterns that influence stress and cortisol (Waladkhani and Hellhammer 2008).

3.2.3. Physical activity

In the natural stress reaction, **energy is mobilized** as preparation for activity in the fight/flight response. Consequently, physical activity would be an ideal stress reaction since it burns the mobilized energy and prevents energy storage. Nevertheless, stress is seldom leading to physical activity in our modern life and the energy is stored again (Tsatsoulis and Fountoulakis 2006).

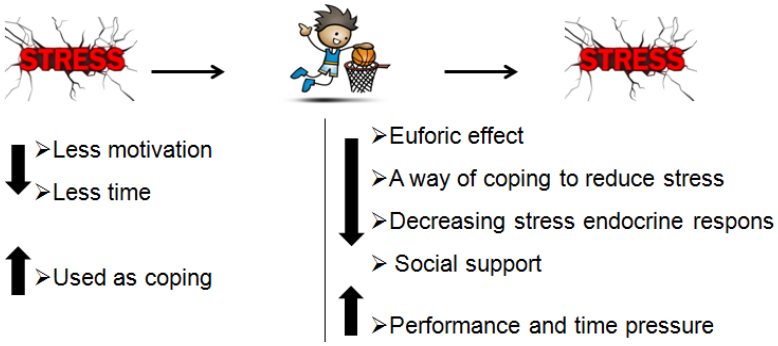


Figure 13: Associations between stress and physical activity.

The hypothesis on the stress-activity relation are shown in Figure 13. Stressed people probably might not have the **motivation or the time** to be active resulting in decreased activity. Nevertheless, physical activity can be used **as a way of coping** by some people, especially those with a high usual level of physical activity (Balantekin and Roemmich 2012, Cairney, Kwan et al. 2013). Remarkably, most research has focused on the effect of physical activity on stress since it distracts from stress, it generates euphoric feeling, it increases social support and even decreases the stress response (Tsatsoulis and Fountoulakis 2006). Nevertheless, physical activity could also increase the stress level if there is high performance and time pressure. Recent reviews in children/adolescents showed a limited effect on stress although this is mostly based on cross-sectional findings (Larun, Nordheim et al. 2006, Biddle and Asare 2011).

Apart from a decreased physical activity level, a simultaneous **increase of screen time** is hypothesized. Indeed, evidence in reviews on child/adolescent populations was even more consistent for an association between high sedentary screen time and poor mental health than for physical activity (Kappos 2007, Biddle and Asare 2011).

3.2.4. Sleep

Sleep is the **metabolic antagonist of stress** because of their opposite effects on heart rate, blood flow and hormones (Buckley and Schatzberg 2005, Akerstedt 2006).

Not only sleep duration may be influenced by stress but also **sleep quality** such as long sleep latency, many awakenings or short time in important sleep phases such as the rapid eye movement (REM) and slow wave sleep (SWS) phase (Kim and Dimsdale 2007).

Apart from the fact that stressed people might have a lack of time for long sleep, also genetic, hormonal, neural and psychological mechanisms exist (see Figure 14). On a **hormonal** level, short sleep increases stress hormones and these hormonal changes inhibit sleep and influences important sleep phases. The difficult combination of stress with sleep can be demonstrated by the circadian rhythm of the stress hormone cortisol: cortisol has a peak in the morning when awakening and very low levels in the night when preparing to sleep. On **neural** level, the regulation of sleep, behaviour and emotions are closely related: interactions of sleep loss on amygdala and prefrontal cortex functions (Horne 1993, Gregory and Sadeh 2012) and also anatomical interconnections between different centres of the hypothalamus that regulate sleep and stress (Rolls, Schaich Borg et al. 2010) have been found. Consequently, sleep loss will be associated with deteriorated performance and mood. On **psychological** level, sleep is essential for proper emotion regulation while stress can result in problems to fall asleep due to rumination or next day anticipation. Rarely, sleep related stress is reported: being stressed because of worrying about the sleeping problems (Morin, Stone et al. 1993).

A recent review showed that the sleep-stress relation in children and adolescents is likely bidirectional: most evidence shows that sleep problems or insufficient sleep exacerbate emotional and behavioural difficulties, while mood disturbances and anxiety perhaps compromise sleep patterns. Nevertheless, the field is full of **discrepancies recommending more longitudinal research** in different age groups (Gregory and Sadeh 2012).

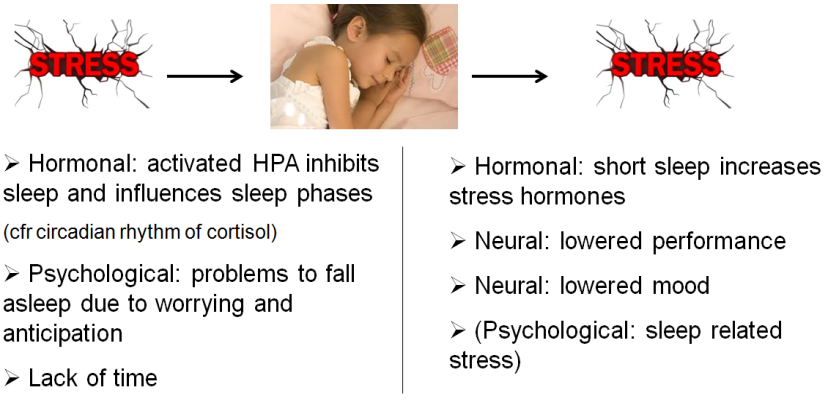


Figure 14: Associations between stress and sleep.
HPA= hypothalamic-pituitary-adrenal axis

4. Lifestyle

4.1. Lifestyle in childhood

In public health, "lifestyle" generally means a pattern of individual practices and personal behavioural choices that are related to elevated or reduced health risk (Kirch 2008). This doctoral thesis will focus on the lifestyle factors diet, physical or sedentary behaviour and sleep. In our westernized countries, lifestyle trends include a significant decrease in outdoor recreation and sleep duration and an increased dependence on electronic media and comfort food. Moreover, these lifestyle factors have been shown to **track into later life** (Thorleifsdottir, Bjornsson et al. 2002, Mikkila, Rasanen et al. 2005, Ashcroft, Semmler et al. 2008, Telama 2009).

For public health, dietary guidelines are formulated in the prevention of chronic diseases (obesity, cardiovascular diseases and diabetes) with a focus on low consumption of energy dense foods like those high in sugar and fat, but also a high consumption of fruit and vegetables (WHO 2003). Nevertheless, these **food recommendations** are not strictly followed in current childhood. In the HBSC study, only 42% of the 11y old children consumed fruit daily, while 18% reported daily soft drink intake (Currie, Zanotti et al. 2009). Apart from the type of foods consumed, **nutrient composition** is an important health determinant. In European children, the fat intake expressed in percent of total energy intake (%E) was above the recommended maximum of 30%E and also the sucrose intake of 15.5%E exceeded the recommendation of less than 10%E (Elmadfa, Meyer et al. 2009). One of the major shifts in dietary lifestyle that took place during the last decades is the increasing consumption of foods and drinks **out of home**. Although half of the families have meals together 5 to 7 nights per week, 31% share 1 to 4 meals together and even 14% do not share any meals together. Children and adolescents that often have a family meal seem to have healthier dietary and eating patterns (Hammons and Fiese 2011). Moreover, the trend for eating out house has been related to higher energy intake and more specifically higher energy from fat (Lachat, Nago et al. 2012). Another unhealthy dietary habit is **meal skipping**. In the HBSC study, on average 29% of the 11y olds skipped breakfast regularly (Currie, Zanotti et al. 2009).

For physical activity and sedentary behaviour in children, recommendations have been formulated with at least **60 minutes mean-to-vigorous physical activity** per day and **not**

more than two hours screen time per day (American Academy of Pediatrics 2001, Strong, Malina et al. 2005). Nevertheless, only 23% of the 11y olds in the HBSC study reported at least one hour of moderate-to-vigorous physical activity daily and even 56% reported two or more hours TV watching daily. Even lower activity levels have been reported in European children between 2 and 9y (15% reaching the recommendation) or between 10 and 12y (4.6% in girls, 16.8% of boys) (Verloigne, Van Lippevelde et al. 2012). Indeed, there is evidence for a decreasing trend in physical activity and fitness an increasing trend in screen time (Dollman, Norton et al. 2005).

Also for sleep, recommendations have been published. Although sleep needs are variable from child to child, around **10.5 hours of sleep** have been recommended in primary school children (National Sleep Foundation). A median sleep around 10 hours/day has been published for European primary school children (Hense, Barba et al. 2011). Apart from sleep duration, also sleep quality is important. Night-time fears, worry and anxiety about daytime matters may cause difficulties in getting to sleep or staying asleep. Also clinical sleep problems have been diagnosed in children such as the restless legs syndrome and sleep-disordered breathing (Mindell and Owens 2003, National Sleep Foundation 2004).

Importantly, all these lifestyle factors have been shown to **influence each other**. Screen time has been related to increased food intake, decreased sleep duration and decreased physical activity (Epstein, Paluch et al. 2002, Chaput, Klingenberg et al. 2011), while short sleep increases energy intake and decreases activity (Patel and Hu 2008, Nishitani, Sakakibara et al. 2012).

4.2. Relevance for the stress-obesity relation

Diet, activity and sleep need to considered when studying the stress-obesity relation because of four reasons. First of all, these lifestyle factors have been reported to be used **as a coping for stress**. In a childhood population, playing, eating, watching TV and relaxing were the most reported stress coping strategies. Next to these, also sleeping and walking/running were reported but less frequent (Taxis, Rew et al. 2004, Chen and Kennedy 2005). Chen et. al. also investigated what children report as the most efficient coping strategies. Drawing, talking and relaxing were reported as the most efficient coping reactions, followed by walking/running, playing, watching TV, eating/drinking and sleeping (Chen and Kennedy 2005). Secondly, these lifestyle factors are important **determinants for childhood obesity** as was discussed in

the previous section (Davison and Birch 2001, Skelton, Irby et al. 2011). Thirdly, lifestyle factors have been hypothesized to be an **underlying path in the stress-obesity relation** (Gatineau and Dent 2011, Pervanidou and Chrousos 2011). Fourthly, lifestyle behaviour might be **targeted to enhance stress resilience** since lifestyle influences the allostatic load (McEwen 2007).

5. Study aim

The ChiBS study (Children's Body composition and Stress) is an observational cohort study that was designed to investigate the relationship between chronic psychosocial stress and changes in body composition (body fat) over a two-year follow-up period (2010-2012) in a non-clinical population sample of young children (5-12 years old). It is hypothesized that the exposure to **chronic stressors may affect children's body composition** in the long-term by promoting body fat increase and the development of obesity. More specifically, this study examines the influence of chronic stress on the evolution of different adiposity parameters longitudinally, taking into account diet, sleep and physical activity as intermediary factors in this relationship. It is hypothesized that chronic stress may promote adiposity directly **through hormonal increase of fat** deposition **and/or indirectly through changes in lifestyle factors** such as the increased consumption of energy dense highly palatable (sugar and fat rich) foods and a deviant eating behaviour, decreased quantity and quality of sleep and a decreased amount of physical activity (with an increase in sedentary screen time), as indicated in Figure 15. Understanding the mechanisms by which stress may predispose to obesity, will elucidate preventive strategies. To accurately measure stress, child- and parent-reported stress questionnaires as well as objective stress biomarkers were used. Besides this major aim, it was also the goal to test the **feasibility and interrelationships of these different stress measurements** in children.

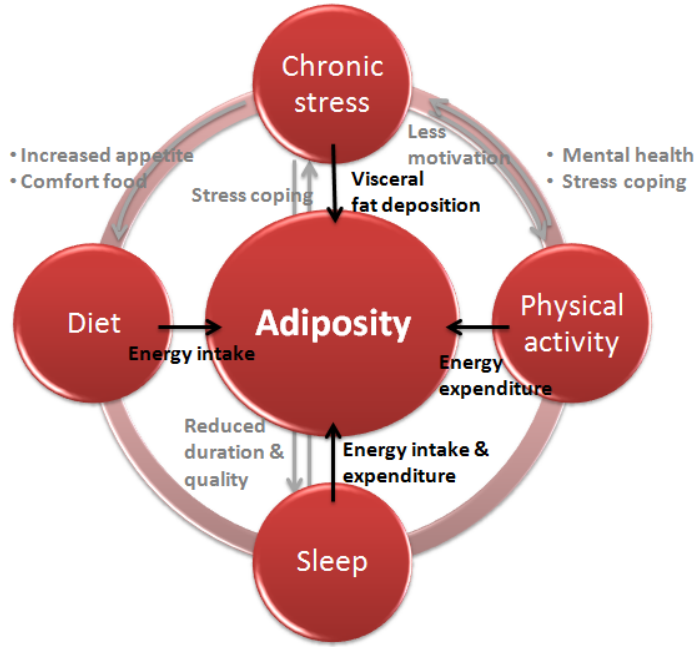


Figure 15: Lifestyle factors involved in the development of adiposity and investigated in the ChiBS study. *Grey arrows indicate the study hypotheses, black arrows show the effect of the four lifestyle factors on adiposity.*

6. Outline of the thesis

A brief discussion of the ChiBS study design, the population sample and the used methods is given in chapter II **"Methodology"**.

Chapter III to V present the different **results**: methodological results (III), lifestyle results (IV) and adiposity results (V). Each chapter is based upon an independent paper which has been published or submitted in a peer-reviewed journal.

A first methodological aim was to test the interrelationships of the **different stress measurements.** This is studied in chapter III.1 to III.4. Chapter III.1 and III.2 describe two biomarkers that represent the most important stress-pathways: salivary cortisol measurements representing the HPA stress system and HRV measurements representing the ANS. Only recently, these two biomarkers have been increasingly used in children. At the start of our study, there was no consensus on reference concentrations, salivary cortisol sampling confounders/compliance and determinants. Chapter III.3 and III.4 explore the interrelationship between the different stress measurements. Not only the relation of the biomarker with the stress questionnaire data was tested but also the interrelationship of the two biomarkers mutually.

A second methodological interest was on **body composition measurement**. In this study, an advanced method of body composition determination was used: the BOD POD® device based on ADP technology. The BOD POD has a quick, comfortable, automated, non-invasive and safe measurement process. Nevertheless, large-scale epidemiological studies are often restricted to routine measurements because of the logistic (immobile device) and budgetary constraints of more advanced technologies like ADP. Therefore, we tested the relative validity of routine anthropometry like BMI, skinfold thickness, body circumferences and foot-to-foot bioelectrical impedance in chapter III.5.

The third and central aim was to study the **relation of stress with adiposity**. As stated in Figure 10, two main pathways in the stress-adiposity relation were hypothesized: a direct hormonal pathway (by cortisol) and an indirect behavioural pathway (by lifestyle). Consequently, also the stress-lifestyle relation was analysed. Diet (pattern and behaviour), physical activity, sedentary behaviour and sleep (duration and quality) were considered as lifestyle factors that simultaneously might be influenced by stress and promote adiposity.

Chapter IV.1 and IV.2 present the cross-sectional relations of stress (questionnaire data and salivary cortisol) with diet as main lifestyle factor. Since saliva was exclusively collected at baseline, the cortisol-diet relation could only be tested cross-sectionally. Chapter IV.3 tested the association of sleep duration and especially sleep quality with adiposity. After all, sleep duration has been increasingly linked to the aetiology of obesity, but less research has focused on children's sleep quality. Subsequently, the stress-lifestyle relation was evaluated longitudinally in chapter IV.4. Finally, the cross-sectionally and longitudinally (bidirectional) stress-adiposity relation is shown in chapter V.1 and V.2. In studying the effects of stress on adiposity, mediation and moderation by cortisol and lifestyle was tested to verify the hypothesized direct and indirect pathway in the stress-adiposity relation.

This thesis ends with a **general discussion** in chapter VI. After discussing the main results and the methodological considerations, the relevance for public health and directions for future research are suggested.

II.

METHODOLOGY

This chapter is based on the paper:

Michels N, Vanaelst B, Vyncke K, Sioen I, Huybrechts I, De Vriendt T, De Henauw S, 2012. Children's Body composition and Stress – the ChiBS study: aims, design, methods, population and participation characteristics. *Archives of Public Health*, 70 (17).

1. Recruitment of participants

Approach and enrolment of the participants for the baseline survey of ChiBS (February 2010) was largely simplified by integrating this study in the **IDEFICS project** (Identification and prevention of dietary- and lifestyle-induced health effects in children and infants). The IDEFICS project was a European project funded by the European Sixth Framework Programme (Ahrens, Bammann et al. 2011) that commenced in September 2006 and ran to February 2012. This **multi-centre** project consisted of two elements. The first element was a prospective cohort study in a large diverse sample of children to investigate the aetiology of diet- and lifestyle-related diseases and disorders with a strong focus on overweight and obesity. Second, the IDEFICS study developed, implemented and evaluated community-oriented intervention programmes for primary prevention of obesity in a controlled study design. For this purpose, an intervention region and a control region were selected in eight European countries (Belgium, Cyprus, Estonia, Hungary, Germany, Italy, Spain and Sweden). The baseline measure in 2007-2008 was performed in about 16000 2-9 year old children and was followed by an intervention. A second survey reassessed the children two years later (school year 2009-2010) to determine the longitudinal associations and to assess effects of the intervention.

For the **baseline survey of ChiBS (T1)**, measurements were started during the IDEFICS follow-up survey of the Belgian control region (i.e. the municipality Aalter) **in February – May 2010**. All participating children in the control region (N=761) were eligible to join the ChiBS study. Children of the intervention region were not included since it was assumed that intervention effects have influenced the lifestyle of those children. At baseline, the children were between 5 and 11 years old (first four years of primary school). IDEFICS measures were done at school. For extra ChiBS measures, parents had to come to the municipal sports park in Aalter outside school hours (Wednesday afternoon, weekends and holidays). Their parents were individually contacted by providing a letter via the schools, wherein they were informed and invited to let their children participate in the ChiBS study. Parents were asked to sign a consent form, in which the option was offered to participate in the full ChiBS programme or in a selected set of measurement modules.

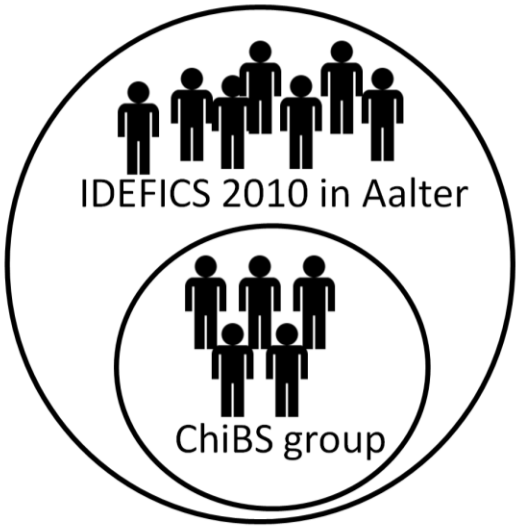
A second and third survey module of the ChiBS study was conducted in **February-April 2011 (T2)** and **February-April 2012 (T3)** to fully cover primary-school age. For both follow-up surveys, the parents of participating children were contacted telephonically and were asked

to sign a new consent form, in which options for all separate examination modules were offered. The fieldwork for T2 and T3 was conducted at the municipal sports park of the city Aalter (permanent localisation of measurement device i.e. BOD POD®).

Figure 16 schematically presents the timeline of the ChiBS project and the corresponding measurements and examinations of each survey period. The **IDEFICS measures** that were used for the T1 ChiBS study and that were reassessed during the ChiBS follow-up are dietary intake, physical activity and sleep duration (Ahrens, Bammann et al. 2011). Also background variables such as socio-demographics (e.g. parental education, income, place of birth) and medical conditions were collected in the IDEFICS study. The **extra measures for ChiBS** concern stress-assessment (questionnaires and biomarkers), BOD POD adiposity measurement, sleep quality and eating behaviour. Most measurements were performed in all three waves (2010, 2011 and 2012). Some measures were only performed at baseline: salivary cortisol (because of high costs and logistics) and sleep quality (because of the logistics). Two wave data is available for eating behaviour, daily hassles/uplifts, waist circumference, sleep duration and accelerometry.

2. Participation numbers

Participation numbers for the different examination modules are shown in Figure 16. Numbers for some examination modules were lower because of several reasons: 1) parents could chose in what examination modules they participated, 2) some questionnaires were not fully completed, 3) HRV data must meet certain quality criteria, 4) some cortisol level analyses were unsuccessful. **At baseline, 523 children** of the 761 IDEFICS children participated in the ChiBS survey. This was a participation proportion of 68.7%. It should be considered that IDEFICS participants belonged already to a motivated sample of the population since they decided to participate in IDEFICS. Nevertheless, participation numbers were lowered by the high burden due to the large battery of measurements and the need to make an appointment at the sports park. Of these 523 children, **453 participated again in 2011** (T2) and **330 in 2012 (T3)**. From T1 to T3 a drop-out of 37% was seen. Nevertheless, we succeeded in including **326 children that participated in all 3 waves** and an extra 129 children in two of the three waves. Participants with and without complete data for two or three waves were compared using Little's Missing Completely At Random test (Little 1988). A non-significant χ^2 test statistic suggested that the data is missing completely at random.



	baseline 2010 N=523		1st follow-up 2011 N=453		2nd follow-up 2012 N=330	
	cross-sectional		longitudinal			
Stress biomarkers	Salivary cortisol	N=439				
	HRV	N=460	HRV	N=412	HRV	N=311
Stress questionnaires	CLES-C	N=491	CLES-C	N=418	CLES-C	N=320
			Daily uplifts/hassles		Daily uplifts/hassles	
	Emotions		Emotions		Emotions	
	Coping		Coping		Coping	
	SDQ		SDQ		SDQ	
Antropometry	BOD POD	N=497	BOD POD	N=453	BOD POD	N=330
	BMI	N=523	BMI	N=453	BMI	N=330
	Skin folds	N=515				
	Waist circumference	N=518			Waist circumference	N=329
Diet	FFQ	N=375	FFQ	N=341	FFQ	N=311
			DEBQ	N=438	DEBQ	N=324
Physical activity	Activity questionnaire	N=488	Activity questionnaire	N=397	Activity questionnaire	N=308
	Screen time	N=483	Screen time	N=391	Screen time	N=323
	Accelerometry	N=366			Accelerometry	N=252
Sleep	Sleep duration	N=333			Sleep duration	N=325
	Sleep quality	N=253				

Figure 16: Timeline of the ChiBS project, corresponding measurements of each survey period and participation numbers

BMI= body mass index, CLES-C= Coddington Life Events Scale for children, DEBQ= Dutch Eating Behaviour Questionnaire, FFQ= Children's Eating Habits Questionnaire-Food Frequency Questionnaire, HRV= heart rate variability, SDQ= Strengths and Difficulties Questionnaire

3. Population description

In view of a number of budgetary and logistical advantages, it was decided to embed this study within the European IDEFICS project, as already mentioned above. Table 3 shows the children's and parental socio-demographic characteristics of **participants to the baseline ChiBS study compared to ChiBS non-participating children**. We did not find significant socio-demographic differences between ChiBS participants and ChiBS non-participants. This indicates that there was **no further participation bias introduced** by the subjects included in the ChiBS study in comparison with the existing IDEFICS cohort. Table 3 however shows that ChiBS non-participants were younger than ChiBS participants. The differential attrition for 5-year olds (as indicated in Table 3) can be explained by the introduction of eligibility criteria: an age cut-off (i.e. first year of elementary school) for stress questionnaires was introduced since kindergarten children cannot complete the questionnaires in a reliable way. As we recruited the children at classroom-level and not at individual level, the participation of 6 year olds may also be artificially distorted as some children of the last kindergarten-year may already be 6 years old and were therefore not included in this study. The goal was thus to cover all primary school ages, by examining the first to fourth class-year (commonly covering the ages between 6 and 10) at baseline and by examining third to last year of primary school (normally covering the ages between 8 and 12) at the final follow-up. In this way, children of **all years of primary school were covered throughout this study**.

We also compared the ChiBS participants that participated in both 2010 and 2012 (n=330) and **those that dropped-out after 2010** (n=193) to detect a certain selection bias in the follow-up study (see Table 3). No differences were seen on age, sex, BMI and income. Nevertheless, parents of those that dropped out had a **lower education level and more non-traditional family structure**. It might be that parents with a higher education level are more interested in scientific studies and that non-traditional family structures might thwart the organisational arrangements to make an appointment each year. The children that participated again in 2012, **did not differ on their stress score** compared to those that dropped out between 2010 and 2012 (p=0.826 for negative emotions; p=0.406 for SDQ total problems and p=0.144 for the CLES negative events score over the last year). Also no difference was found in body fat%, diet, sleep duration and physical activity (data not shown), although those that dropped out had a **higher screen time** (p=0.016).

Table 3: Socio-demographic characteristics of non-participants and ChiBS participants in 2010 (T1) and 2012 (T3).

	ChiBS T1 nonparticipants		ChiBS T1 participants		X^2-test nonparticipants vs T1	ChiBS T3 participants		X^2-test T3 vs T1[†]
	%	N=228	%	N=523	p-value	%	N=330	p-value
child characteristics								
sex					0.634			0.797
male	51.3	117	49.3	258		49.1	162	
female	48.7	111	50.7	265		50.9	168	
age (years)					<0.001			0.109
5	55.7	127	0.4	2		0	0	
6	19.3	44	11.9	62		2.4	8	
7	7.5	17	24.7	129		12.7	42	
8	6.6	15	26.6	139		15.2	50	
9	7.0	16	24.9	130		20.3	67	
10	3.1	7	11.1	58		24.2	80	
11	0.9	2	0.6	3		20.6	68	
12	0	0	0	0		4.5	15	
BMI					0.550			0.841
underweight	11.8	27	13.6	71		15.2	50	
normal	82.0	187	79.3	415		76.4	252	
overweight	5.7	13	5.5	29		7.6	25	
obese	0.4	1	1.5	8		0.9	3	
parental characteristics								
household income	(35 missing)		(110 missing)		0.377	(33 missing)		0.082
low to low/medium	5.7	11	5.1	21		3.4	8	
medium	45.1	87	41.6	172		40.7	121	
high/medium	27.5	53	26.2	108		25.3	75	
high	21.8	42	27.1	112		30.6	91	
education (ISCED)	(12 missing)		(30 missing)		0.163	(10 missing)		0.002
level 1	0.9	2	0.6	3		0	0	
level 2	2.8	6	0.6	3		1.6	5	
level 3	29.2	63	26.2	129		28.4	91	
level 4	19.9	43	21.9	108		15.0	48	
level 5 or higher	47.2	102	50.7	250		55.0	176	

family structure	(13 missing)		(32 missing)		0.066	(11 missing)		0.007
traditional	87.4	188	81.9	402		86.5	276	
non-traditional	12.6	27	18.1	89		13.1	43	
migrant status								
father migrant	2.2	5	1.1	6	0.770	0.6	2	0.390
mother migrant	3.1	7	3.4	18	0.274	2.7	9	0.501

BMI= body mass index with cut-offs by the International Obesity Task Force classification; household income categories based on national statistics; ISCED= International Standard Classification for EDucation (1 'primary education', 2 'lower secondary education', 3 'upper secondary education', 4 'post-secondary non-tertiary education', 5 'tertiary education'); traditional family structure: children living with both biological parents; non-traditional family structure: all other family structures. † T3 participants were compared with those that participated in T1 but dropped-out afterwards.

To further examine the representativeness of our study population, the socio-demographic characteristics of the study sample was **compared with those of the general population in Flanders**. This socio-demographic information was obtained through consulting statistics of the Flemish authorities and 'Child and Family' (Studiedienst Vlaamse regering 2007, Kind en Gezin 2010). Parents of the children participating to **ChiBS were higher educated and less often of migrant origin** compared to the general Flemish population (47.8% versus 31.7% of ISCED 5 or higher; 3.4% versus 6.4% of migrants; respectively). Additionally, a traditional two-parent family structure is more prevalent in ChiBS participants compared to the general Flemish population (76.9% versus 66.1% of traditional family structures, respectively). The **overweight and obesity percentages in ChiBS were low** compared to the most recent reference data available for Flemish children. The Flemish Growth Survey 2002-2004 (in 16000 Flemish children and adolescents) demonstrated overweight and obesity percentages of 14.2% and 3% for female participants respectively, and 11.8% and 2.6% of overweight and obesity for male participants respectively (2-18 years old) (Roelants, Hauspie et al. 2009). Although these results suggest a low representativeness, it should be noted that these findings are inherently related to the **overall higher socio-demographic characteristics of the municipality Aalter** compared to the general Flemish population in 2010 (Studiedienst Vlaamse regering 2012): 1.9% versus 6.4% migrants, 3.9% versus 6.8% unemployment rate, 17154 euro versus 16599 euro year income per inhabitant, 1.4% versus 8.6% births in underprivileged families.

4. Methods

The following paragraphs describe all the methods, questionnaires and measurements that were performed in the framework of the ChiBS study.

4.1. Stress questionnaires

Stress arises when the demands of a situation exceed an individual's ability to cope and resolve the problematic situation, resulting in emotional and behavioural disturbances (McCance, Forshee et al. 2006). To cover the different aspects of stress, negative events, emotions and problem behaviour were examined by questionnaires. Children were individually interviewed by a trained researcher to obtain information about their life events, daily hassles and uplifts, coping strategy and emotions. Parents were asked to report on their child's behavioural and emotional problems. Only children from primary school were eligible to fill in the questionnaires (not kindergarten children).

4.1.1. Life events (child-reported)

The 'Coddington Life Events Scale' for children (CLES-C) (Coddington 1972, Coddington 1999) was purchased (reliability: r=0.69; construct validity=0.45). The English questionnaire was translated professionally into Dutch using a translation and back-translation process to ensure identical meaning. This validated 36-item questionnaire measured the frequency and timing of events in the last year relevant for this age group and resulted in a 'life change units' score for the time periods 0-3, 0-6, 0-9 and 0-12 months ago. To limit recall bias and to increase the accuracy of reporting in time, interviewers used calendar events such as birthdays, summer holidays and Easter (Garrison, Schoenbach et al. 1987). Moreover, drawings and pictures were used to clarify some of the most difficult events, such as 'juvenile court', 'respect' etc. Apart from the total event score (both negative and neutral events), also a score for exclusively negative life events was calculated. Negative events included familial issues (e.g. divorce), school issues (e.g. failing a grade), social issues (e.g. moving), criminal issues (e.g. juvenile court), economical issues (e.g. job loss of parents) and illness/death (of child/family/friend/pet).

4.1.2. Daily hassles and uplifts (child-reported)

The children's daily hassles (CHS) and daily uplifts (CUS) scales of Kanner et al. (Kanner, Coyne et al. 1981) contain 25 hassles and 25 uplifts, respectively. Also for children as young

as 5 and 6 years old, an internal consistency of 0.85 was shown and daily hassles correlated with parental reported behavioural problems (Creasey, Mitts et al. 1995). Hassles refer to irritating, frustrating or distressing demands that characterize everyday transactions with the environment. Uplifts refer to positive experiences such as the joy derived from friendship, relief at hearing good news and so on. Children were asked to check which hassles and uplifts occurred during the last month. Furthermore, they were asked to rate whether they felt 'not bad', 'sort of bad', or 'very bad' as a result of the hassle and whether they felt 'OK', 'sort of good' or 'very good' as a result of the uplift. Both a total frequency, a frequency of higher intensity hassles and uplifts ('sort of bad' or 'very bad' and 'sort of good' or 'very good', respectively) and an intensity score can be calculated.

4.1.3. Emotions (child-reported)

Children were questioned about their feelings in general. Since it concerned a young childhood sample, a short and easy-to-understand questionnaire was chosen (see Annex). As in the study of Zimmer-Gembeck (Zimmer-Gembeck, Lees et al. 2009), the feelings anger, anxiety, sadness and happiness were rated on a 0 to 10 Likert-scale (0 'not at all' to 10 'very strong'). To help the young children understand these distinct feelings, pictures of a social skills training game for very young children were displayed next to the question (Dupondt 1992). These basic emotions are understandable for infants and children (Flavell 1999) and can therefore uncomplicatedly be used in our population. The sum of the negative emotions (anger, anxiety, sadness) was validated against the well-know PANAS-C questionnaire (Laurent, Catanzaro et al. 1999) that can be used for children of at least 9 years old. Moderate correlation (Spearman $r=0.48$ $p<0.001$) with the negative affect score of the PANAS-C questionnaire was found in a sample of 153 children that were between 9-13y old at follow-up.

4.1.4. Coping (child-reported)

The children were asked what they usually do when they are confronted with problems or when they are upset by using an 8 item-questionnaire, with 'never' (score 0), 'sometimes'(score 1) or 'often' (score 2) as response alternatives (see Annex). This questionnaire was previously used in the CASE-study (Child and Adolescent Self-harm in Europe) (Madge, Hewitt et al. 2008) and translated into Dutch and substantially pilot-tested for a population of Belgian adolescents (Portzky, De Wilde et al. 2008). Although no psychometric data on this coping questionnaire was available for our age group, other coping

questionnaires have been used with children's self-report (Blount, Simons et al. 2008) and acceptable repeatability was shown in 5 to 6-year old children with open-ended questions (r between 0.67 and 0.77) (Creasey, Mitts et al. 1995). The answers were classified as emotion- versus problem-focused coping, based on the transactional model of Lazarus and Folkman (Folkman, Lazarus et al. 1986). Emotion-focused coping (items 2, 3, 4, 6 and 7) is aimed at regulating emotional stress while problem-focused coping (items 1, 5 and 8) deals with the problem and makes changes in the disturbed and stress-inducing person-environment relationship. A coping index was calculated: the problem-focused score (ranging from 0 to 10 after rescaling) minus the emotion-focused score (ranging from 0 to 10). Theoretically, the coping index could range between -10 and 10, with higher scores showing problem-focused dominance.

4.1.5. Emotional and behavioural problems (parent-reported)

Parents were asked to complete the standardized 'Strengths and Difficulties Questionnaire' (SDQ) (reliability: ICC=0.80; concurrent validity: r=0.70) (Goodman 1997), reporting the emotional and behavioural problems of their child over the past 6 months. For each of the 25 statements, parents could answer: 'not true' (0), 'somewhat true' (1) and 'certainly true' (2). The statements were divided in 5 subscales of 5 items each: emotional problems, conduct problems, hyperactivity-inattention behaviour, peer problems, and prosocial behaviour. Subscale scores were computed by summing scores on relevant items (after recoding reversed items). Higher scores on the prosocial behaviour subscale reflect strengths, whereas higher scores on the other subscales reflect difficulties. Questions on the hyperactivity-inattention behaviour scale were not used in the IDEFICS study, as such this subscale was not available at baseline.

4.2. Stress biomarkers

Besides using questionnaires to analyse stress, also biomarkers were applied. In analysing stress by biomarkers, the two most important stress-pathways were distinguished: cortisol measurements as a marker of the hypothalamic-pituitary-adrenal stress system and heart rate variability as a marker of the autonomic nervous system.

4.2.1. Salivary cortisol

Saliva was collected into Salivette synthetic swabs especially designed for cortisol analysis (Sarstedt, Germany) (see Figure 17). The participants were asked to collect saliva during two consecutive weekdays at four time points: immediately after waking (T0), 30 minutes after waking (T30), 60 minutes after waking (T60) and in the evening between 7 and 8 PM (Tev). The time point of awakening was defined as 'immediately after opening the eyes'. All the weekdays were included, with Friday being less frequent (2%). Weekdays were chosen above weekend days since they represent more the daily life and they allow better standardisation of collection hours (more parental supervision and less dispersion of awakening hour). To standardize sample collection, sampling instructions for the children and their parents were provided in a manual: the children were asked to sample when healthy and on a normal day; to respect the time points; not to eat, drink or brush their teeth in the hour before collection; to avoid physical activity two hours before sampling; and to avoid caffeine-rich drinks and minimize medication on the sampling days. The manual also provided a stepwise description for the sampling and storage. The parents were also asked to fill in a checklist about instruction compliance and other factors: (1) awakening time, collection hours, health, physical activity, caffeine consumption and medication on the days of collection and (2) the compliance to food restriction and teeth brushing one hour before the sampling. The checklist was based on the one published by Hanrahan et al. (2006). As we found that parental reported 'time non-compliers' showed lower cortisol concentrations and less pronounced CAR than 'time compliers' (Michels, Sioen et al. 2012), a quality control was executed by excluding morning samples collected more than 5 minutes different from the requested time point and evening samples not collected between 7 and 9 PM (271 out of 3290 samples). Furthermore, samples of corticosteroid-users were also excluded (5 children).

The samples were picked up at school in the morning after the two sampling days and were kept cool during transport. The same day, the Salivettes were centrifugated for 5 minutes at 3000 RPM (Jouan CR412 centrifuge) and the filtrates were stored at -20 °C. Salivary cortisol was assayed within the first month after collection in the routine laboratory of the Ghent University Hospital on a Modular E 170 immunoanalyser system (Roche Diagnostics, Mannheim, Germany) by the Roche Cobas Cortisol assay. The precise working mechanism and features of this analysis technique are described elsewhere (van Aken, Romijn et al. 2003). This competitive electrochemiluminescence immunoassay with a measuring range between 0.018-63.4 µg/dl (0.49-1748.95 nmol/L) had an inter-assay coefficient of variation of

3.9% and an intra-assay coefficient of variation of 1.9% while for samples near the lower detection limit the coefficients of variation were respectively 12.7% and 10.2% (based on laboratory's internal quality assessment). The cortisol concentrations from the analysis (µg/dL) were converted into SI units (nmol/L) by multiplying the values with a conversion factor of 27.586 (Young and Huth 1998).

Figure 17: Collection method for salivary cortisol (the salivette) and heart rate variability (a chest belt).

4.2.2. HRV

Inter-beat RR-intervals (RRI) were recorded at a sampling rate of 1000 Hz for 10 min in supine position with an elastic electrode belt (Polar Wearlink 31) (see Figure 17). This low-cost device has been validated against an electrocardiogram device in children (Gamelin, Berthoin et al. 2006). Children were asked to refrain from strenuous physical activity on the measurement day. The heart rate belt was fixed around the chest and measurements started when the signal stabilized. To ensure a non-stressful situation, measurements were done in a quiet, closed room in a familiar building (the local sports park) child by child. Comforting music was played and a blindfold was provided if desired. The child was encouraged to breathe normally and not to speak or move during the measurement. Further data processing was performed with the free professional HRV Analysis Software of the University of Kuopio, Finland (Niskanen, Tarvainen et al. 2004). Low-frequency (LF) and high-frequency (HF) bands were analysed between 0.04-0.15 Hz and 0.15-0.4 Hz as default (Task Force of ESC/NASPE 1996). The RR series were detrended using the Smoothness priors method with $\alpha = 300$, and a cubic interpolation was done at the default rate of 4 Hz. The middle 5 min were manually checked for quality (no large RRI outliers, RRI equidistance, and Gaussian RR distribution), and if necessary, another appropriate 5-min interval was selected.

For time domain methods, the mean RRI (mRR), the standard deviation of the normal RRI (SDNN), the root mean square of successive differences (RMSSD) and the percentage of consecutive normal RRI differing more than 50 ms (pNN50) were determined. For the

frequency domain methods, both parametric autoregression (AR) as well as nonparametric fast Fourier transform (FFT) models were used. FFT spectrum parameters were calculated with the Welch's periodogram method using a standard 50% overlap Hanning window as pre-processing technique and finally an integration (area under the curve). AR spectrum coefficients were calculated using the forward-backward linear least squares method. The AR model was not fixed, but personally optimized (model order ranged between 12 and 28, mean 17) since the previously recommend fixed order 16 (Boardman, Schlindwein et al. 2002) was sometimes far from ideal. The AR model choice was based on the following criteria visible in our program: (1) no third peak after the LF, (2) the intersection between HF and LF as close as possible to 0.15Hz, (3) little overlap between LF and HF. Using AR and FFT, the power of LF and HF bands in absolute and normalized units [nu] (LF or HF divided by "total power minus very low frequency power"*100) and the LF/HF ratio were determined. RMSSD, pNN50 and HF reflects the vagal parasympathetic activity and the LF/HF ratio is assumed to represent the sympathovagal balance. More difficult is the interpretation of LF and SDNN, since it contains both parasympathetic and sympathetic contributions.

4.3. Anthropometry

4.3.1. ADP with BOD POD

Body volume was measured by ADP (BOD POD®, Software version 4.2.4, Life Measurement Inc, Cranlea and Co, Birmingham, United Kingdom) using standardized procedures (McCrory, Gomez et al. 1995). ADP is similar in principle to hydrostatic (or "underwater") weighing. The obvious difference is that air is more convenient and comfortable than water, so that ADP provides a much easier and safer testing environment. Children had to refrain from physical activity and food two hours before the measurement. The BOD POD® was calibrated daily and at each measurement according to the manufacturer's guidelines. For the measurement, children had to sit in the device for about 1 minute in tight-fitting bathing suit with swim cap to rule out air trapped in clothes and hair (see Figure 18). If the first two readings for body volume differed by more than 150ml, a third measurement was taken and the two values that were closest and within the criteria for agreement were averaged. Thoracic gas volume was predicted by the software with a validated child-specific equation (Fields, Hull et al. 2004). Fat percentage was calculated using the up-to-date child-specific conversion factors reported by Wells (Wells, Williams et al. 2010).

Figure 18: The BOD POD device for fat percentage determination.

4.3.2. Routine anthropometry

All anthropometric measurements were carried out by three trained researches to enhance intra- and inter-observer reliability (Stomfai, Ahrens et al. 2011). The children were measured barefooted in underwear and /or T-shirt.

Weight was measured in fasting status with an electronic scale to the nearest 0.1 kg. *Height* was measured with a telescopic height measuring instrument (SECA 225, UK) to the nearest 0.1 cm. The *BMI* z-score was obtained by calculating the BMI (*BMI=weight(kg)/height(m)²*) and adjusted for age and sex using British 1990 growth reference data (Cole, Freeman et al. 1998). Overweight was determined by the International Obesity Task Force classification (Cole, Bellizzi et al. 2000).

Leg-to-leg impedance (ohm) was measured with the electronic TANITA® BC 420 SMA scale (prototype adapted to the small foot size of children). As impedance is dependent on length of the conductor, an impedance index reflecting the fat-free mass was defined as *impedance index=height²/impedance*. To reflect fat-mass, 'weight minus impedance index' was calculated.

Skinfold thicknesses (mm) were measured twice on the right side of the body to the nearest 0.2 mm with a skinfold calliper (Holtain, UK, range 0-40 mm) according to the international standards for anthropometric assessment (Marfell-Jones, Olds et al. 2006) and the mean of both measurements was calculated. The triceps skinfold was taken halfway between the acromion process and the olecranon process at the back side of the arm. The subscapular

skinfold was measured 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body. If the first and second measurement of the skinfolds differed more than 2 mm, a third measurement was performed.

Circumferences (cm) were measured once with an inelastic tape (Seca 200, precision 0.1 cm, range 0-150 cm) with the subject in a standing position. Circumference measurements were performed at the following three sites: 1) mid-upper arm (MUAC) relaxed arm, halfway between the acromion process and the olecranon process; 2) waist, halfway between the top of the iliac crest and the lower coastal border (10^{th} rib); 3) hip, at the maximum extension of the buttocks.

In all analyses, body composition parameters were examined as continuous variables, such as body fat percentages, fat free mass percentages, BMI z-scores etc., instead of comparing obese versus non-obese groups.

4.4. Lifestyle factors

4.4.1. Food Frequency Questionnaire (FFQ) (parent-reported)

The FFQ is a screening instrument to investigate food consumption frequency and behaviours associated with obesity and general health in children. This 43 food-item-containing instrument was developed and reproducibility (test-retest r= 0.32-0.76 for separate items) was tested within the IDEFICS project (Lanfer, Hebestreit et al. 2011). It is used as a screening instrument to investigate dietary habits and food consumption frequency in children. Parents were asked to report on the frequency of their child's consumption of each of the preselected food items (43 items) during the preceding 4 weeks using one of the following response options concerning the intake frequency of each food item: 'never/less than once a week' (0/week), '1-3 times a week' (2/week), '4-6 times a week' (5/week), '1 time per day' (7/week), '2 times per day' (14/week), '3 times per day' (21/week), '4 or more times per day' (30/week) or 'I have no idea'.

Frequencies of intake were assessed without quantifying portion sizes. To identify dietary patterns, four 'food indices' on dietary pattern were calculated by summing up the frequency of separate food items: a food index for 'sweet foods' (sweet drinks, jam, honey, sweet breakfast cereals, sweet snacks), 'fatty foods' (fried potatoes, chocolate- or nut-based spreads, high fat dairy, mayonnaise and mayonnaise-based products, cheese, fat meat preparations,

butter, high fat snacks), ‘snacks’ (chocolate and chocolate bars, candy, biscuits, cake, ice-cream, chips, savoury pastries) and also a healthy food index for ‘fruit and vegetables’ (fruit, freshly squeezed fruit juice, vegetables).

4.4.2. Dutch Eating Behaviour Questionnaire (DEBQ) (child-reported)

In the DEBQ, a 33-item questionnaire (test-retest r= 0.87-0.90; Cronbach’s alpha=0.72-0.89), three types of eating behaviour can be identified in children: eating in response to negative emotions (emotional eating), eating in response to the sight or smell of food (external eating) and eating less than desired to lose or maintain body weight (restrained eating). In all three types of eating behaviour, the appropriate self-regulating mechanism of food intake is diminished or lost. Children could answer the questions on their usual behaviour with ‘never’ (1), ‘almost never’ (2), ‘sometimes’ (3), ‘often’ (4) or ‘very often’ (5) as response alternatives (van Strien, Frijters et al. 1986).

4.4.3. Physical activity and sedentary behaviour (parent-reported)

Parents were asked about the usual physical activity and screen time of their child at each measuring wave. The sum of usual hours of physical activity outdoors and at sports club per week were used as a measure of physical activity. The reported usual number of screen time hours per week (e.g. television and computer time) was used as a measure of sedentary behaviour.

4.4.4. Physical activity and sedentary behaviour (objectively measured)

The children were asked to wear an Actigraph® accelerometer for five consecutive days in 2010 and 2012 during waking hours on an elastic belt on the right hip. Activity counts were stored at 15 second intervals. To be included in the data analysis, children had to wear the accelerometer for at least 8 hours per day, not exceeding 18 hours per day and for at least 3 days. Data was classified into four bands of physical activity using the cut-off points of Evenson depending on the counts per minute: sedentary (0-100), light (100-2295), moderate (2296-4011) and vigorous activity (≥4012) (Trost, Loprinzi et al. 2011). Moderate and vigorous activity were summed to calculate moderate-to-vigorous physical activity. These accelerometer counts were expressed using percentages by further dividing them by total recording time to correct for wearing time.

4.4.5. Sleep duration (parent-reported)

Parents reported in 2010 and 2012 the usual time of the child going to bed in the evening and getting up in the morning on weekdays and weekend days.

4.4.6. Sleep quality (objectively measured)

Sleep quality was measured by actigraphy. Children had to wear an accelerometer (Actigraph®, Pensacola, FL, USA) at the wrist to detect movement during sleep. Parents had to fill in a sleep diary with the hours of awakening and going to sleep to calculate the reported sleep duration. At least 3 nights including both week and weekend days and a complete sleep diary filled in by the parents were necessary to interpret the data (Littner, Kushida et al. 2003). Raw accelerometer data was sampled in 1-minute epochs and was converted to sleep parameters with the Actilife-software of the accelerometer. Activity patterns were visually checked to ascertain that the accelerometer was worn during the night. Movement at a threshold level for a period of time was scored as "awake" based on the built-in Sadeh scoring algorithm that has previously been validated against polysomnography with agreement rates between 91 and 93% (Sadeh, Sharkey et al. 1994). The following sleep parameters were obtained: (1) sleep quantity or reported sleep duration or time between reported 'time in bed' and 'time out bed', (2) sleep onset or time point of falling asleep, (3) sleep latency or time between 'time in bed' and sleep onset, (4) minutes scored as wake after sleep onset (WASO), (5) actual sleep duration or corrected sleep duration without sleep latency and WASO, (6) sleep efficiency or the proportion of actual sleep over the total time spent in bed.

4.5. Parental education

Socio-economic status was represented by the highest parental education (maximum of both parents) according to the International Standard Classification of Education (ISCED) (Unesco 2010). Due to low variation, the variable was further categorized in two levels of education (low/medium vs. high), with ISCED levels 0-3 being defined as low/medium education and level 4 and 5 being defined as high education (=tertiary education).

4.6. Ethical approval

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital. From the parents, a written informed consent was obtained, and from the children an verbal assent.

III.

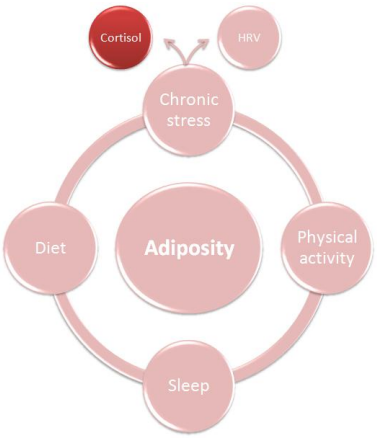
METHODOLOGICAL RESULTS

1.

Children's morning and evening salivary cortisol: pattern, instruction compliance and sampling confounders.

Michels N, Sioen I, De Vriendt T, Huybrechts I, Vanaelst B, De Henauw S,

Hormone Research in Paediatrics 2011; 77: 27-35.



The right thing at the wrong time
is the wrong thing.

ABSTRACT

BACKGROUND: Salivary cortisol has been widely used to assess childhood stress. Yet, there is no consensus on reference concentrations, awakening response, guideline compliance and contribution of sampling factors to the variation in children's salivary cortisol levels.

METHODS: Samples were collected from 444 Belgian children participating in the ChiBS study (5-11 years old) on two consecutive weekdays at four moments: awakening, 30 minutes later, 60 minutes later and in the evening. A checklist requested awakening time, collection hours and guideline compliance.

RESULTS: Percentile values were determined. Mixed model analyses revealed that age, time compliance and awakening time contributed significantly to the variance in cortisol levels. In only 52.5% of the children a cortisol morning increase was observed. Participants with no morning increase showed higher awakening but lower post-awakening concentrations on that day and the morning response showed a small negative correlation with the time lag between first and second sampling.

CONCLUSION: This study emphasizes the importance of excluding extreme time deviation and correcting for age and awakening time. Appearance of a cortisol morning increase was only found in approximately half of the children, suggesting the absence of the cortisol awakening response as general characteristic. Although, this could partially be explained by poor time compliance.

INTRODUCTION

The study of cortisol patterns has become a major aspect of stress research. Under basal conditions, cortisol secretion has a circadian rhythm with the lowest level in the first half of the night and a peak in the early morning. Apart from this circadian rhythm, there is also a cortisol awakening response (CAR) showing a quick cortisol increase within 20 to 30 minutes after awakening in the morning (Fries, Dettenborn et al. 2009). Salivary cortisol is especially useful for studying the CAR in children as, in comparison to blood sampling, saliva sampling is stress-free and allows multiple sampling throughout the day in a natural environment (Gozansky, Lynn et al. 2005, Levine, Zagoory-Sharon et al. 2007).

As a result, there has been growing interest in the CAR in children (Pruessner, Wolf et al. 1997, Rosmalen, Oldehinkel et al. 2005, Osika, Friberg et al. 2007). Still, research is needed to obtain representative and comparable cortisol concentration data. A first issue is the standardization of sampling schedules over different studies. A large amount of studies has not been sampling the CAR (i.e. sampling at awakening and fixed times afterwards) but only once at a fixed hour (e.g. between 7 and 8 AM) (Jessop and Turner-Cobb 2008). Secondly, consensus has not been reached on reference values for children's salivary cortisol (Jessop and Turner-Cobb 2008). In addition, the current literature gives mixed results with some studies suggesting similar CAR in both children and adults (Pruessner, Wolf et al. 1997, Wust, Wolf et al. 2000) and others suggesting possible age differences (Kudielka, Broderick et al. 2003). Consequently, the CAR phenomenon in children requires more standardized research. To attribute salivary cortisol correctly as a stress biomarker, the biological and methodological variation need to be considered (Hansen, Garde et al. 2008, Adam and Kumari 2009, Kudielka and Wust 2010), also in children (Hanrahan, McCarthy et al. 2006, Jessop and Turner-Cobb 2008). Nevertheless, disagreement remains on which external (e.g. brushing teeth and awakening time) and internal (e.g. sex and age) factors actually contribute to this variation.

The purpose of this paper is (1) to describe and examine the stability of morning and evening salivary cortisol values and the CAR phenomenon in children, as well as parent-reported instruction compliance, (2) to examine the influence of specific internal (age, sex) and external (routine action compliance, time compliance and awakening time) factors on children's salivary cortisol values in a large population sample and (3) to determine the study

population's cortisol ranges. This investigation is a progress in the development of salivary cortisol reference values for children, in the elucidation of specific factors influencing these values, and in the methodological standardization by formulating crucial guidelines. As this paediatric research project is based on a large sample size, the methodological findings are of particular interest in the future research on children's salivary cortisol.

METHODS

Participants, general procedures and context

In the ChiBS study, 454 children collected saliva samples for cortisol analysis. These children (49.3% male) were between 5 and 11 years old with a mean of 8.4 years. This methodological paper is the first output of the ChiBS project. Detailed aims, design, methods, population and participation characteristics can be found in chapter 2 "Methodology".

Salivary cortisol

Saliva was collected at home via Salivette synthetic swabs (Sarstedt, Germany) immediately after wake up (T0), 30 minutes after wake up (T30), 60 minutes after wake up (T60) and in the evening between 7 and 9 PM (Tev). More details on sampling and analyses can be found in chapter 2 "Methodology".

Data analysis

All statistical analyses were performed using SPSS/PASW version 18 (IBM Corp, NY, USA). Due to a skewed distribution, cortisol concentrations were logarithmically transformed by the natural logarithm and median values with the interquartile range are reported. To analyse the CAR, two parameters were computed: the area under the curve with respect to the ground (AUCg) and the absolute increase of cortisol (AINC), summarizing respectively the total hormonal secretion (intensity) and the time course (sensitivity) of salivary cortisol. AUCg was calculated as the total area under the curve between T0 and T60 (Pruessner, Kirschbaum et al. 2003), while AINC was calculated as the concentration difference between peak (maximum of T30 and T60) and T0. The use of these two parameters instead of the actual morning samples gives the opportunity to reduce the amount of analyses and to simplify the interpretation (Fekedulegn, Andrew et al. 2007). The two parameters were first calculated from the non-transformed concentrations and were log-transformed afterwards.

Time compliance in the morning was defined for each time point as whether or not being sampled in a range of 5 minutes before or after the exact time points (being T0, T30 and T60). Time compliance in the evening was defined as whether or not being sampled between 6.30 and 8.30 PM, giving a margin of 30 minutes around the instructed time point (between 7 and 8 PM). For evening samples, time compliance can be handled with less accuracy as cortisol values change more slowly than in the morning. Time compliance was categorized in three groups: too early, in time and too late.

Only a limited number of people reported noncompliance for the other instructed factors: (1) food intake or teeth brushing in the hour before collection or (2) caffeine consumption or physical activity two hours before sampling or (3) use of influencing medication (i.e. corticosteroids and some other medications) on the sampling day. Compliance for these factors was summarized into one variable, 'routine action compliance'. Noncompliance for at least one of these five factors was sufficient to be classified as routine action noncompliant. Medications that are considered to influence cortisol values have been documented previously (Hanrahan, McCarthy et al. 2006).

Independent contributions to cortisol variation of age, sex, time compliance, routine action compliance and awakening time were analysed by means of mixed models. Sensitivity tests were done to analyse the effect of excluding cases based on the mixed model results, i.e. exclusion of cases being non compliant for time (defined as above-mentioned). This was done by means of an ANCOVA analyses, to compare the means in cortisol concentration for 'total population (included and excluded)' versus 'included population' after correction for age and awakening time.

The LMS method (Cole and Green 1992) was used to generate smoothed percentile values across age for each of the time points and the morning-to-evening ratio. The LMS Chartmaker Pro software (version 2.3) uses cubic splines to fit smoothed *L* (skewness), *M*, (median) and *S* (coefficient of variation) curves across each age category by maximized penalized likelihood. Q tests and detrended Q-Q plot were used to asses goodness-of-fit and normality.

Within- and between-person variation, S_w (square root of estimated within-person variance), and S_b (square root of estimated between-person variance) were estimated, using analysis of variance (one-way GLM) with the Minimum Norm Quadratic Unbiased Estimation method. Coefficients of Variation (CV) were calculated as: CV_w=[S_w/mean concentration]x100; CV_b=[S_b/mean concentration)]x100. Cortisol concentrations in the samples over the two days were compared with a paired t-test. In this analysis, samples of one individual on the two days

were considered as repeated measurements. Pearson correlations were used to investigate correlations between two continuous variables. T-test or Pearson χ^2- test were used when analysing the relationship between categorical data and respectively continuous or categorical data. In analyses where age and awakening time were considered as categorical, two groups were created by dividing the population in a group below mean and a group above mean. Statistical results with $p<0.05$ were considered as significantly different.

RESULTS

Descriptive analysis

From a total of 454 children who finally collected and returned their saliva samples, 10 children did not return the checklist and thus only data from 444 children were analysed. From this group, only 350 (78.8%) children collected all eight samples. In total, 3376 samples were collected. Most missing samples were the last two samples from the second day. Parents indicated that not the sampling itself but the specific timing and the several restrictions were perceived as difficult. Despite the low detection limit of our assay and the use of synthetic swabs specifically developed for cortisol analysis (Groschl, Kohler et al. 2008), 48 (1.42%) samples had a cortisol concentration below the detection limit and 38 (1.26%) samples did not have the required volume to perform the analysis. Of the 48 samples that were below the detection limit, 45 (93.75%) were evening samples. This resulted in a final amount of 3290 valid samples for further statistical analysis.

Salivary cortisol values showed great variance. Of 3290 samples, 37 (1.1%) were higher than three standard deviations from the mean. These children reported no medication intake nor showed these samples visible blood contamination. This outlier-pattern was not consistent over the two days, but in 40% of the cases it was consistent over several sampling times. Cortisol median and ranges for the four consecutive time points were 11.92 [1.66-100.41] (T0), 12.25 [2.07-115.86] (T30), 7.92 [0.74-65.10] (T60) and 1.68 [0.5-17.93] nmol/L (Tev). For the four time points, the CV_w were 36.2%, 33.6%, 43.0% and 41.5% respectively and the CV_b were respectively 49.8%, 52.2%, 49.9% and 102.7%. Pearson correlation coefficients between two days for the four time points were respectively 0.399, 0.336, 0.498 and 0.509 (all having $p<0.001$). The median AINC was 0.48 nmol/L (-3.30 to 4.06), resulting in a median increase of 4.31% (-26.75% to 39.93%). The median AUCg was 22.19 (17.39 to 28.58). A Pearson correlation coefficient of 0.451 for AUCg ($p<0.001$) and 0.043 for AINC ($p<0.05$)

was found between the two days. After excluding all noncompliers, correlation coefficients did not differ drastically.

Significant partial Pearson correlation coefficients ($p<0.001$) were found between the AUCg and the AINC ($r=0.267$), between the evening sample and the three morning samples ($r=0.347$, 0.262 and 0.319 respectively) and between the AUCg and the evening sample ($r=0.359$). No significant correlation could be found between the AINC and the evening sample ($r=0.011$, $p=0.102$).

Cortisol awakening response

When analysing the three morning samples, a typical morning increase or CAR was not always observed since in 47.5% of the samples a decrease is found. When excluding the time noncompliers, a similar percentage was found. Only 35% of the samples met the previously published criterion of a minimum increase of 2.5 nmol/L to be a 'responder' (Wust, Wolf et al. 2000). The AINC even changed sign between both days in 41.2% of the children and only 31.4% of the children showed a positive AINC on both days. Therefore, the samples were also analysed by comparing the group with morning increase and the group without morning increase based on the AINC value (Figure 19). When comparing these two groups, no significant differences were observed for sex, age, awakening time, evening concentration, routine compliance, the delay between awakening and T0, the delay between T0 and T30 and family structure. Nevertheless, the group with a positive AINC had a lower cortisol concentration at T0 ($p<0.001$) and higher concentration at T30 and T60 ($p<0.001$) compared to those with a negative AINC (Figure 19). Similar results were obtained when using the AINC as a continuous variable, except that the continuous AINC also showed a significant correlation with the time delay between T0 and T30 ($r=-0.076$, $p=0.038$). Excluding the participants using corticosteroids or other possible affecting medication did not change the results at all.

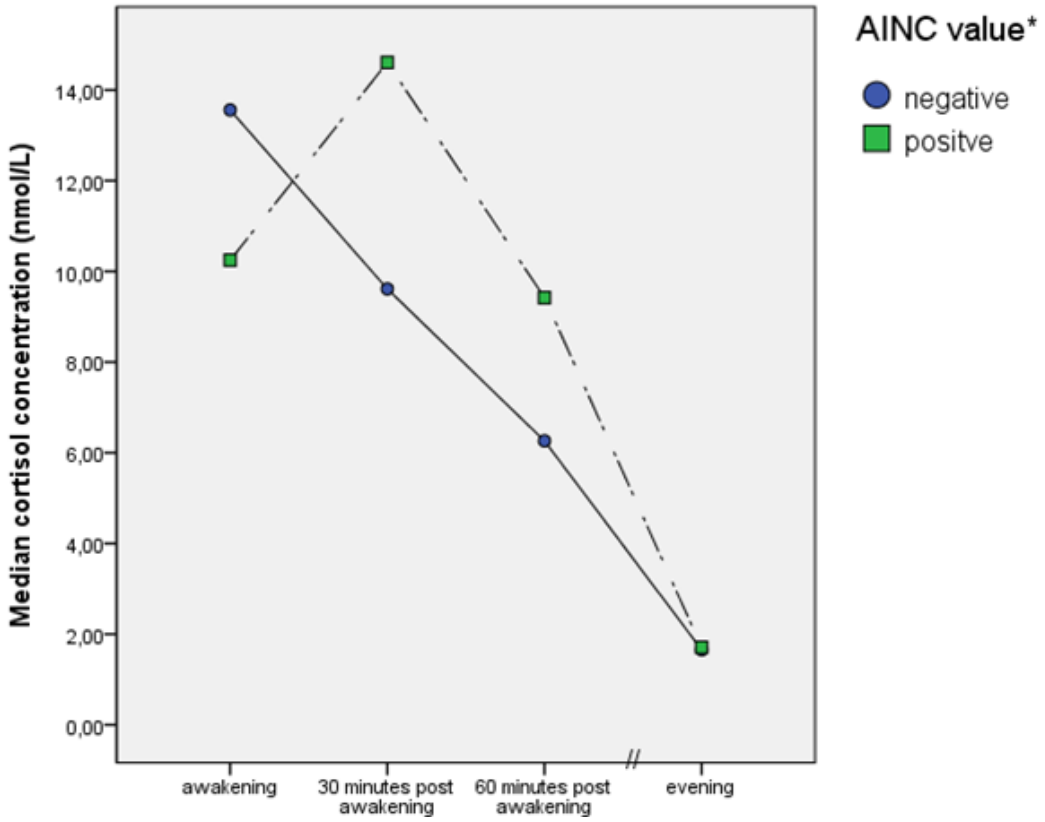


Figure 19: Difference in cortisol pattern (median concentrations) between the days with and without increasing morning cortisol.

**AINC= absolute morning increase of cortisol*

Parent-reported compliance

Table 4 gives an overview of the percentages of noncompliers for time and routine action (n=3290). Additionally, since some of these actions might influence several samples of one day (eating, caffeine and teeth brushing) or even all samples of both days (medication use), compliance numbers for these actions were given by day and by child respectively. 2413 (73.3%) samples were fully compliant for these stringent guidelines. The mean awakening time was 0656h (SD=31 minutes) with a mean of 2.1 minutes (SD=5.0) time between awakening and T0, 30.2 minutes (SD=7.9) between awakening and T30 and 60.7 minutes (SD=8.8) between awakening and T60. The evening samples were taken on average at 1949h (SD=37 minutes). The participants that were noncompliant for the routine actions also had a greater chance to be noncompliant for time ($\chi^2(1)=7.25$, p=0.009). No correlations were found between compliance and external factors or family structure. Corticosteroids and 'other affecting medication' were used by 16 (3.5%) and 5 (1.1%) children, respectively. The

corticosteroids were either nasal or aerosol medication and 90% of 'other affecting medication' group concerned Rilatine®.

Table 4: Parent-reported noncompliance to the protocol

	Noncompliers	
Samples (n=3290)	**n**	**%**
Physical activity	102	2.9
Caffeine	18	0.5
Eating	70	2.1
Teeth brushing	21	0.6
Time	458	13.9
Routine action	259	7.9
	Noncompliers	
Children (n=444)	**n**	**%**
Corticosteroids	16	3.5
Other affecting medication	5	1.1
Time compliance waking	**n**	**%**
In time	762	91.9
Too late	67	8.1
30 minutes post awakening	**n**	**%**
Too early	31	3.6
In time	810	93.5
Too late	25	2.9
60 minutes post awakening	**n**	**%**
Too early	40	4.7
In time	753	88.9
Too late	54	6.4
Evening	**n**	**%**
Too early	22	2.7
In time	672	82.7
Too late	119	14.6

Influence of internal and external factors on salivary cortisol

Table 5 shows the results of the mixed model analyses investigating the independent contributions of age, sex, time compliance, routine action compliance and awakening time to the variance in salivary cortisol. Interaction factors for the different parameters (also sex*age) did not give significant results and were eliminated in further analyses. The cortisol values and consequently also the AUCg increased significantly with age on all time points. Sex did not have any contribution, even when performing separate analyses for two age groups (data not shown). Apart from age, time compliance and awakening time were important contributors: sampling too late or awakening later resulted in lower cortisol concentrations (Table 5). Action noncompliance resulted in lower concentrations on T30 and T60, but this was not significant when excluding participants using corticosteroids. This change to non-significance was not created by lack of power as action compliance maintained significance by random exclusion of a similar percentage. After excluding sex and action noncompliance, results remained almost the same (data not shown).

The sensitivity test by excluding time noncompliers resulted in a different mean cortisol for 'total population (included and excluded)' versus 'included population' for T30 and AUCg ($p<0.05$) with higher values in the compliant population.

Table 5: Contribution of internal and external factors to cortisol variance by mixed model analysis

β-values for the factors at different time points						
	Waking	**30 min post**	**60 min post**	**Evening**	**AUCg**	**AINC**
Intercept	2.486***	2.679***	2.293***	0.571***	3.271***	4.294***
Sex [§]	-0.078	0.066	0.002	0.021	-0.053	0.008
Age	0.047**	0.044*	0.058**	0.086***	0.046**	-0.001
Action compliance[#]	-0.086	-0.219*[$]	-0.178*[$]	-0.022	-0.149	-0.032
Time compliance						
Time lag T0	0.005				-0.001	-0.001
Time lag T30		-0.007**			-0.008**	-0.001*
Time lag T60			-0.004*		0.001	
Time lag evening				-0.002*		
Awakening time	-0.091**	-0.159***	-0.149***	0.082	-0.152***	-0.011

** significant at the 0.05 level (2-tailed); ** significant at the 0.01 level (2-tailed); *** significant at the 0.001 level (2-tailed); [§] The reference category is male; [#] The reference category is compliant; [$] Not significant after excluding participants using corticosteroids; AUCg= area under the curve with respect to the ground of the three morning samples; AINC= absolute increase of cortisol in the morning*

Salivary cortisol percentile values

As demonstrated by mixed model analyses, time noncompliers and corticosteroid users should be excluded. After exclusion, percentile values were computed for the different years of age (5 to 11) as a guide for endocrinologists (see
Table 6). No stratification for gender was done as no gender differences in cortisol values were observed.

Table 6: Age-specific L, M, S and percentile values of salivary cortisol (nmol/L).

Age (years)	L	S	3rd	10th	25th	50th (M)	75th	90th	97th
Waking									
5	0.266	0.253	6.579	7.954	9.528	11.320	13.347	15.629	18.185
6	0.227	0.340	5.406	7.016	8.973	11.329	14.135	17.452	21.339
7	0.175	0.433	4.357	6.065	8.293	11.154	14.786	19.340	24.994
8	0.128	0.466	4.154	5.863	8.155	11.194	15.176	20.342	26.979
9	0.125	0.446	4.587	6.368	8.725	11.814	15.820	20.968	27.526
10	0.136	0.430	5.095	7.002	9.497	12.725	16.860	22.108	28.711
11	0.118	0.408	5.978	8.035	10.693	14.097	18.423	23.880	30.714
30 minutes after waking									
5	0.735	0.333	5.458	7.961	10.695	13.630	16.744	20.020	23.445
6	0.490	0.402	4.287	6.395	8.932	11.901	15.305	19.147	23.427
7	0.224	0.469	3.817	5.578	7.913	10.944	14.807	19.654	25.651
8	0.138	0.483	4.133	5.941	8.393	11.671	15.998	21.643	28.927
9	0.058	0.476	4.734	6.601	9.146	12.595	17.243	23.474	31.783
10	0.138	0.508	4.144	6.080	8.753	12.382	17.242	23.665	32.055
11	0.310	0.553	3.158	5.296	8.268	12.226	17.328	23.736	31.615
60 minutes after waking									
5	0.263	0.530	2.422	3.841	5.796	8.401	11.780	16.069	21.411
6	0.189	0.538	2.292	3.532	5.268	7.640	10.814	14.981	20.367
7	0.084	0.544	2.220	3.288	4.810	6.954	9.942	14.067	19.709
8	0.014	0.547	2.486	3.598	5.196	7.491	10.779	15.484	22.204
9	-0.034	0.535	2.963	4.188	5.945	8.475	12.132	17.445	25.197
10	-0.029	0.508	2.983	4.151	5.795	8.116	11.404	16.078	22.745
11	-0.014	0.481	3.347	4.596	6.321	8.706	12.007	16.584	22.939

Evening									
5	-0.639	0.484	0.559	0.691	0.885	1.187	1.704	2.729	5.370
6	-0.566	0.505	0.601	0.756	0.982	1.337	1.942	3.119	5.972
7	-0.470	0.532	0.594	0.763	1.013	1.406	2.073	3.332	6.147
8	-0.375	0.556	0.597	0.787	1.069	1.513	2.254	3.603	6.369
9	-0.279	0.573	0.643	0.870	1.210	1.740	2.606	4.110	6.929
10	-0.184	0.572	0.661	0.914	1.291	1.867	2.772	4.245	6.742
11	-0.088	0.577	0.696	0.992	1.430	2.086	3.085	4.625	7.040
Morning-to-evening ratio									
5	0.316	0.513	2.630	4.207	6.332	9.095	12.588	16.902	22.133
6	0.221	0.544	2.388	3.759	5.677	8.283	11.740	16.229	21.956
7	0.038	0.567	2.384	3.529	5.192	7.596	11.052	15.997	23.034
8	0.007	0.586	2.206	3.269	4.841	7.159	10.576	15.606	23.004
9	0.141	0.609	1.741	2.796	4.358	6.617	9.819	14.270	20.355
10	-0.007	0.601	2.142	3.190	4.755	7.096	10.601	15.854	23.735
11	-0.279	0.578	2.946	3.993	5.567	8.029	12.074	19.135	32.455

L (skewness), M (median), S (coefficient of variation) and percentiles with LMS method

DISCUSSION

The first objective of this paper was to describe the salivary cortisol values and the CAR phenomenon in a large paediatric study. The results showed a high within- and between-person variability and significant weak positive between-day correlation coefficient, reflecting the known large biological variation in salivary cortisol (Schulz and Knabe 1994). The first two morning samples (T0 and T30) had the largest variability as they were taken at the time of rapid rise to morning peak. The very weak positive correlation for AINC between two days confirms the need of multiple day sampling for AINC (Hellhammer, Fries et al. 2007). Only a weak positive correlation could be found between the two morning summary parameters AUCg and AINC, supporting the fact that AUCg and AINC reflect another aspect of the awakening response (Fekedulegn, Andrew et al. 2007).

The CAR rise was only present in 35% of the children, compared to 42% and 75% in previous studies, respectively in children between 10 and 12 (Rosmalen, Oldehinkel et al. 2005) and in adults (Wust, Wolf et al. 2000, Eek, Garde et al. 2006). Previous literature could not yet fully

explain the phenomenon of CAR-nonresponders, but are inclined to point more to confounders like time compliance to partially explain the negative awakening response (Kudielka, Broderick et al. 2003, DeSantis, Adam et al. 2010). Using the parent-reported sampling times, no difference was found between the positive and negative AINC group in time compliance. Nevertheless, there was a small negative correlation between the AINC and the time lag T0-T30. When having a long time period between the first and second sampling, the peak at 30 minutes post-awakening could have been missed and could have resulted in a negative AINC. Furthermore, we observed that the children with negative AINC had a higher awakening and lower post-awakening concentration. This was also demonstrated by Dockray et al. (2008) and could be a reflection of a possibly delayed actual sampling by which the increase is missed.

Our instructions resulted in a good compliance. As a result, using an elaborated manual can already minimize the variation in sampling conditions. Although, we have noticed difficulties in standardizing the evening sampling time. In children, sleeping time varies largely by age resulting in difficulties to sample between 1900h and 2000h with at least a one hour lag after dinner. Sampling just before sleeping could possibly create the opportunity for a one hour lag after dinner. but sampling times could still vary largely (Raikkonen, Matthews et al. 2010).

The second objective of this paper was to examine the influence of specific internal and external factors on children's salivary cortisol.

The observed positive association between cortisol values and age in this particular age class was not artificially induced as age did not show any correlation with other factors (e.g. compliance). In contrary, the contribution of age to cortisol variance could theoretically be explained by the further development of the adrenal cortex causing increased glucocorticoid hormones during the adrenarche (Jessop and Turner-Cobb 2008). Although some studies reported higher morning concentration in girls (Netherton, Goodyer et al. 2004, Rosmalen, Oldehinkel et al. 2005, Sondeijker, Ferdinand et al. 2007), we found no contribution of sex which could possibly be attributed to our young age category.

Later awakening time resulted in lower morning cortisol concentrations. These results are in accordance with previous studies in adults (Edwards, Evans et al. 2001, Kudielka, Broderick et al. 2003) and children (Freitag, Hanig et al. 2009, Raikkonen, Matthews et al. 2010) reflecting a common control of sleep status and HPA activity in the hypothalamus.

Time noncompliers showed lower cortisol concentrations except for T0. The noncompliance in these studies could largely be explained by deviation from the awakening definition (immediately after opening the eyes) (Kudielka, Broderick et al. 2003, Dockray, Bhattacharyya et al. 2008, DeSantis, Adam et al. 2010). This unintended and unreported time lack could possibly create an artificially absence of time compliance contribution to the first morning sample. In the absence of objective time recording, one should emphasize the importance of time compliance in the manual and use stringent criteria for exclusion afterwards. Taking into account the sensitivity tests, samples not following our strict definition of time compliance should be excluded in further analyses.

All restricted routine actions have been shown to increase cortisol concentrations (Hansen, Garde et al. 2008). Surprisingly, in our study routine action noncompliance did not have an overall effect or even contributed to lower concentrations on T30 and T60. The unexpected lowering effect on T30 and T60 disappeared after excluding corticosteroid-using participants. This emphasized the importance of excluding corticosteroid-using participants. Following arguments try to explain the absence of an increase: (1) Evening physical activity was previously shown to influence cortisol values only in boys (Kertes and Gunnar 2004) and also non-mentioned physical activity (e.g. playing in the garden) could have influenced cortisol values; (2) Teeth brushing can cause micro trauma and thus serum cortisol contamination, but that is only in minor cases the fact (Granger, Cicchetti et al. 2007); (3) The consumed amount of food and caffeine could perhaps be too low to have an impact or only certain nutrients could have an influence (Gibson, Checkley et al. 1999).

Methodological issues and limitations

The strength of our study is the large-scale paediatric measurements of both morning and evening saliva collected during two consecutive days per child. Nevertheless, some sampling limitations should be mentioned. First, information related to sleep and awakening (sleep duration, sleep quality, spontaneous versus forced awakening and the amount of light) and pubertal stage was not reported. Second, it was not possible to examine the seasonal variation as we only sampled during 4 months, but explorative analyses investigating the effect of the sampling month did not indicate a general trend (data not shown). A third limitation is the lack of transferrin measurements to examine blood contamination. Although visible contamination was not present in high cortisol samples, falsely high cortisol values could not be excluded, as would be the case with transferrin analysis. Furthermore, sampling more days

is recommended especially for AINC but this could potentially lower protocol compliance and raise laboratory costs. Finally, there was only a subjective measure of compliance as reported by the parents since a large population sample was included and objective measurement of compliance was therefore impossible. It is probable that noncompliant people are the most likely to report their timing incorrectly, which could be an explanation for the low percentage of CAR observed.

All these limitations are more or less a consequence of the large population scale of the study. We made use of an existing cohort of children for whom a lot of other relevant epidemiological information was collected. Nevertheless this restricted the time scheme for collection and the amount of extra measurements.

CONCLUSION

This large scale methodological study showed that children's salivary cortisol values, especially the absolute increase in the morning, had a rather poor intra- and inter-individual stability over time, emphasizing the need for multiple day sampling. A morning cortisol increase was observed in only 52.5% of the children but this could partially be explained by time noncompliance. The total morning increase was independent of sex or age.

In general, salivary cortisol values in the present study were dependent of the children's age, parent-reported time compliance and awakening time, which emphasizes the importance of using an elaborated manual and checklist. It is important to promote compliance and to exclude large deviations from the time schedule. Furthermore, children's salivary cortisol concentrations should be corrected for age and awakening time. After exclusion of time noncompliers and corticosteroid users, age-specific percentile values were computed as a guide for endocrinologists.

2.

Determinants and reference values of short-term heart rate variability in children.

Michels N, Clays E, De Buyzere M, Huybrechts I, Marild S, Vanaelst B, De Henauw S, Sioen I

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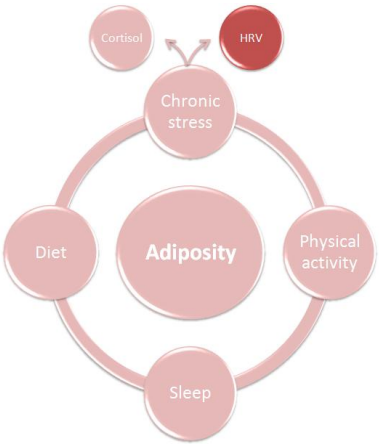

Personally, I need

a high level of physical fitness

in order to feel at ease.

ABSTRACT

INTRODUCTION: This paper provides age- and sex-specific percentile values for short-term heart rate variability (HRV) data in children by time domain and two frequency domain methods. Furthermore, the frequency domain methods will be compared and HRV determinants will be determined.

METHODS: In 460 children (5-10y), 5-minute HRV measurements in supine position were undertaken with Polar chest belts. The data were manually edited and processed with time and frequency domain methods. Interchangeability of two frequency domain methods was investigated using Bland-Altman analyses and intraclass correlation. Age, time point, physical activity (accelerometry), physical fitness (cardiopulmonary fitness, upper and lower limb muscular fitness) and body composition (body mass index, fat%, fat and fat free mass) were analysed as determinants using multiple regression analysis stratified by sex.

RESULTS: Sex- and age-specific percentile values were produced. Overall, girls had lower HRV. Age-related parasympathetic increases and sympathetic decreases were seen with sometimes age-related year-to-year wave-like changes in boys. The time point of recording had limited influence on HRV. Of the lifestyle related factors, fatness (only 7% overweight) was not associated with HRV but fat free mass, physical activity and in particular physical fitness (over and above activity) had a favourable association by increased parasympathetic activity. Furthermore, the quick and frequently used fast Fourier transform method was not interchangeable with autoregression.

CONCLUSION: Future HRV studies in children should consider age, sex and physical fitness and should take into account the non-interchangeability of the frequency domain methods.

Introduction

Heart rate variability (HRV) is defined as the variability of the distance between consecutive R peaks of the electrical heart beat signal (caused by polarization and depolarization of the heart muscles) as measured with an electrocardiogram. The R wave is the first positive deflection of the signal after the P-wave. Consequently HRV is increasingly used as a quantitative marker of the autonomic nervous system i.e. the sympathetic and vagal parasympathetic innervations of the heart (SA and PA, respectively) (1996). These changes reflect the heart's ability to respond to physiological and environmental stimuli. Subsequently, a reduction of HRV (i.e. reduced PA with or without increased SA) is a pathway of increased morbidity and mortality (Thayer, Yamamoto et al. 2010). Apart from its original clinical use as risk marker for cardiovascular mortality, a reduction of HRV has also been observed in non-cardiac pathologies such as stress-induced conditions (Chandola, Heraclides et al. 2010). HRV analysis has increasingly been used in child populations in which HRV analyses showed moderate-to-high reproducibility (Dietrich, Rosmalen et al. 2010). In contrast to long-term recordings (24h), short-term recordings have the advantage of being rapidly obtainable under standardized conditions.

Several physiological factors are known to influence HRV. Age and sex are well-described in adults (Umetani, Singer et al. 1998) with decline over age and lower values in women. However, there are conflicting data in children: (i) no sex difference (Goto, Nagashima et al. 1997, Fukuba, Sato et al. 2009), an overall sex difference (Faulkner, Hathaway et al. 2003) or an age- and measure- dependent sex difference (Silvetti, Drago et al. 2001, Galeev, Igisheva et al. 2002) have been observed as well as (ii) no age difference (Faulkner, Hathaway et al. 2003, Fukuba, Sato et al. 2009), an increase until 6 or 9 year with a decrease or stagnation afterwards (Finley and Nugent 1995, Goto, Nagashima et al. 1997, Massin and vonBernuth 1997, Silvetti, Drago et al. 2001) or more wave-like age-related year-to-year changes (Galeev, Igisheva et al. 2002). Therefore, establishment of age- and sex-specific percentile values is definitely needed.

Associations of HRV have been described with physical activity (Nagai and Moritani 2004, Gutin, Howe et al. 2005, Buchheit, Platat et al. 2007, Krishnan, Jeffery et al. 2009) and body composition (Rabbia, Silke et al. 2003, Nagai and Moritani 2004, Gutin, Howe et al. 2005, Kaufman, Kaiser et al. 2007) in children. Association with physical fitness has rarely been studied (Brunetto, Roseguini et al. 2005, Gutin, Howe et al. 2005) with conflicting results

(only one study showed enhanced HRV), notwithstanding a strong association with cardiovascular risk factors (Hurtig-Wennlof, Ruiz et al. 2007).

Furthermore, some important methodological issues remain to be addressed. First of all, large-scale studies often spread their fieldwork over a long period of the day because of logistic restrictions, but without correction for the time point of registration. Nevertheless, diurnal rhythms of HRV have also been shown in children over a 24h period (Massin, Maeyns et al. 2000). Secondly, two different models have been used in the frequency domain: nonparametric methods such as fast Fourier transform (FFT) and parametric methods such as autoregression (AR). Some studies suggested their non-interchangeability (Fagard, Pardaens et al. 1998, Badilini, Maison-Blanche et al. 2000, Chemla, Young et al. 2005, Pichon, Roulaud et al. 2006), but this has not been confirmed in children. Interchangeability would reduce processing time by applying the easier and quicker FFT method.

In a large healthy child population of 460 subjects, we aimed to provide age- and sex-specific percentile values for an extensive battery of short-term HRV parameters and to test the interchangeability of the two frequency domain methods FFT and AR. Moreover, the contribution of age, sex, time point, body composition, physical activity and particularly fitness was explored.

Methods

Participants and general procedures

Participating children were taken from the baseline Belgian ChiBS survey. Detailed aims, design, methods, population and participation characteristics can be found in chapter 2 "Methodology". The HRV measurements were performed in 475 of the 761 invited Belgian children (62.4% participation rate). No difference in sex, age, body mass index and socio-economic status was observed between participants and non-participants. Exclusion criteria were cardiovascular diseases (1 case), diabetes (0 cases) and HRV measurements of too low quality (14 cases). Finally, 460 children were enrolled for this paper.

Heart rate variability

Five-minute HRV was measured with the electrode belt Polar Wearlink in supine position. Time domain and frequency domain parameters were calculated. For the frequency domain

methods, both parametric AR as well as nonparametric FFT models were used. More details can be found in chapter 2 'Methodology'.

Possible HRV determinants

Children's sex was reported by the parents. *Children's age* was calculated from birth date and examination date. *Time point* was expressed as number of hours elapsed since 9 AM (the earliest measurement).
Physical activity was monitored with accelerometry as described in chapter 2 "Methodology".
Physical fitness was measured with the Eurofit fitness test battery (Council of Europe 1988). Maximal oxygen uptake (VO_{2max}) was estimated through the 20m shuttle test of Léger and Lambert as objective criterion of cardiopulmonary fitness. Handgrip strength was measured by a handgrip dynamometer with adjustable grip (Takei TKK 5401, precision 0.1kg). The sum of right and left arm strength was used. Lower limb muscular fitness was determined by the standing broad jump and the 40m sprint. As both tests were repeated twice, the maximal jump distance (precision 1cm) and the minimal sprint time (precision 0.1s) were used.
Body composition was assessed by age- and sex- specific BMI z-scores and air-displacement plethysmography fat% as described in chapter 2 "Methodology".

Statistical analyses

All statistical analyses were performed using SPSS/PASW version 18 (IBM Corp, NY, USA). Significance was set at $p<0.05$. The positively skewed measurements (HF power, LF power and LF/HF) were log-transformed. Median and interquartile range were given.

Sex differences in HRV were examined by an independent samples t-test. The LMS method (Cole and Green 1992) was used to generate smoothed percentile values across age for all HRV parameters stratified by sex. The LMS Chartmaker Pro software (version 2.3) uses cubic splines to fit smoothed *L* (skewness), *M*, (median) and *S* (coefficient of variation) curves across each age category by maximized penalized likelihood. Q tests and detrended Q-Q plot were used to asses goodness-of-fit.

Agreement between AR and FFT (validity on an individual level) was examined by Bland-Altman analysis. The 95% limits of agreement (LOA) and the presence of a potential upwards or downwards trend across the range of HRV was tested. Trends were significant if there was a significant correlation between the difference in methods and the mean value. As the difference was calculated as 'AR minus FFT', a positive mean difference indicates a FFT

underestimation and an upwards Bland-Altman trend indicates a FFT underestimation with higher HRV values. Single measure intraclass correlation (ICC) was calculated as relative reliability measure using a two-way mixed model with absolute agreement. Interchangeability is defined as ICC>0.75. Furthermore, the correlation, mean difference +/- SD between AR and FFT and its significance by paired t-test with Bonferroni adjustment was given.

To identify determinants, sex and age effects and their interaction were first analysed using two-way ANOVA. The age category '10 years' was merged with the 9-year-olds because of the smaller sample size.

Relationships between HRV parameters and possible determinants were quantified using multiple linear regression stratified by sex. In the basic model, age and time point were entered simultaneously. Then, all other possible determinants (i.e. physical activity, physical fitness and body composition) were entered separately to test their significant contribution after correction for age and time point. Also, their independent contributions were tested after mutual correction (corrected for FFM or for sprint time). Standardized coefficients were recorded. Finally, the multiple linear regression analyses were repeated with correction for mean HR.

Results

Descriptive statistics and percentile values

In total, 460 children (240 boys, 220 girls; 7% overweight) between 5 and 10 years old were included. Participation numbers for the age categories 5, 6, 7, 8, 9 and 10 years old were 72, 62, 89, 113, 84, 40 respectively, evenly distributed over the sexes. Table 7 gives the descriptive statistics and sex differences for all variables. Mean HR was higher in girls, while mRR, SDNN, RMSSD, pNN50, VLF, LF and HF were higher in boys. The sex differences were the same for AR and FFT. Sex differences were also seen in the examined determinants with an overall higher fatness in girls and higher physical fitness and activity in boys. Girls and boys were equally distributed over age and time point ($p>0.05$). Age- and sex-specific percentile values are given in Table 8 and the percentile curves are shown in Figure 20. As no sex differences were seen for LFnu, HFnu and LF/HF, only one set of percentile values was given for both sexes together. As no pattern differences were seen between AR and FFT curves, frequency domain percentile curves were only presented for FFT.

Table 7: Descriptive statistics for HRV measurements and possible determinants of 460 children

	Boys		Girls		Sex difference
	Median	[IQR]	Median	[IQR]	p
mean HR [min^{-1}]	81	[74 - 88]	84	[77 - 91]	0.001*
mean RR [ms]	751	[693 - 824]	725	[662 - 786]	<0.001*
SDNN [ms]	67	[50 - 89]	60	[42 - 78]	0.002*
RMSSD [ms]	73	[54 - 106]	66	[44 - 91]	0.002*
pNN50 [%]	41	[28 - 55]	38	[20 - 52]	0.018*
AR VLF [ms^2]	0	[0 – 0.1]	0	[0 – 0]	0.211
AR LF [ms^2]	521	[274 - 875]	383	[188 - 682]	0.001*
AR HF [ms^2]	421	[240 - 873]	397	[164 - 680]	0.011*
AR LFnu	47	[38 - 58]	48	[39 - 57]	0.785
AR HFnu	44	[33 - 54]	45	[33 - 55]	0.537
AR LF/HF	1.05	[0.72 - 1.72]	1.07	[0.71 - 1.65]	0.495
FFT VLF [ms^2]	23	[11 - 47]	19	[9 - 35]	0.033*
FFT LF [ms^2]	666	[377 - 1327]	550	[262 - 953]	0.005*
FFT HF [ms^2]	1139	[608 - 2012]	967	[441 - 1587]	0.004*
FFT LFnu	38	[30 - 49]	39	[29 - 50]	0.752
FFT HFnu	62	[52 - 70]	61	[50 - 71]	0.753
FFT LF/HF	0.62	[0.42 - 0.95]	0.63	[0.41 - 1.02]	0.867
Time point†	14u	[11u – 15u]	13u	[10u45 - 15u]	0.112
Age (years)	8.1	[6.7 - 9.2]	8.0	[6.8 – 9.0]	0.561
Physical activity					
Counts per minute (n=261)	515.9	[463.2 - 628.4]	474.1	[411.9 - 558.7]	<0.001*
Moderate-to-vigorous activity [min] (n=261)	60.9	[49.9 - 73.4]	44.7	[35.3 - 54.8]	0.005*
Physical fitness					
Arm strength[kgf] (n=314)	27.1	[22.3 - 31.3]	24.9	[20.5 - 28.5]	<0.001*
Maximal jump [cm] (n=315)	125.0	[111.2 – 140.0]	114.0	[104.0 – 129.0]	<0.001*
Sprint time [sec] (n=253)	8.4	[7.8 - 9.2]	8.9	[8.4 - 9.6]	<0.001*
VO_{2max} [$ml.kg^{-1}.min^{-1}$] (n=281)	47.5	[44.8 - 50.6]	45.2	[43.0 – 48.0]	<0.001*
Body composition					
BMI z-score	-0.3	[-0.9 - 0.3]	-0.2	[-0.8 - 0.7]	0.097
Fat percentage	15.7	[12.8 - 19.5]	18.4	[14.8 - 23.7]	<0.001*
Fat free mass [kg]	22.1	[18.8 - 25.1]	21.2	[18.2 – 24.0]	0.012*
Fat mass [kg]	3.9	[3.0 - 5.1]	4.4	[2.4 - 6.7]	<0.001*

*AR= autoregression; FFT= fast Fourier transform; HF= high frequency spectral power; HR= heart rate; IQR= interquartile range; LF= low frequency spectral power; LF/HF= ratio of low frequency power to high frequency power; mRR= mean RR interval; nu= normalized units; pNN50= percentage of consecutive normal RRI differing more than 50 ms; RMSSD= root mean square of successive differences; SDNN= standard deviation of normal-to-normal intervals; VLF= very low frequency spectral power; VO2max= maximal oxygen uptake; * significant difference between girls and boys; † the effective time of the day is shown instead of the number of ours since 9 AM as used in the analyses*

Table 8: Age- and sex-specific percentile values for HRV measurements

Percentile:	2.5	25	50	75	97.5	2.5	25	50	75	97.5
Age			**Boys**					**Girls**		
mRR										
5	520	606	657	713	848	525	602	636	668	726
6	569	664	719	780	921	521	624	672	717	803
7	575	679	736	798	935	536	648	703	759	870
8	580	685	745	808	950	568	673	730	790	919
9	618	715	774	842	1018	607	701	756	816	957
10	666	754	811	883	1101	647	732	782	840	985
SDNN										
5	30	46	57	69	101	13	38	52	67	99
6	32	54	68	84	126	15	39	53	68	101
7	28	52	67	85	128	18	42	57	73	110
8	23	48	63	81	121	21	46	61	78	118
9	21	49	65	83	124	24	47	63	80	122
10	24	54	72	92	136	25	48	63	80	123
RMSSD										
5	26	39	50	64	112	14	40	55	71	107
6	35	61	80	105	181	12	39	55	74	117
7	27	57	77	103	175	14	43	62	85	141
8	19	48	68	92	152	17	47	68	93	159
9	21	52	74	99	164	22	51	72	97	166
10	27	61	84	112	184	27	54	72	96	160
pNN50										
5	5	24	34	44	64	2	16	25	36	63
6	6	28	39	50	74	1	18	29	41	69
7	5	27	38	50	74	1	21	33	46	73
8	4	26	38	49	73	2	24	37	49	76
9	6	29	41	53	78	3	28	41	53	78
10	10	34	46	58	83	7	32	44	56	79
LF AR										
5	97	275	418	608	1158	38	188	344	579	1383
6	107	348	553	832	1665	40	188	342	578	1404
7	84	318	528	821	1723	44	197	358	608	1501
8	59	261	454	729	1604	49	207	376	640	1610
9	49	256	465	773	1777	53	215	389	665	1705
10	45	287	547	939	2255	57	223	402	689	1802
HF AR										
5	37	101	165	269	708	6	101	222	413	1069
6	91	282	481	802	2109	12	128	271	498	1293
7	72	264	468	796	2082	21	163	331	598	1556
8	46	206	381	661	1713	32	197	386	687	1783
9	38	227	439	774	1981	45	228	431	755	1955
10	31	271	552	988	2491	60	256	468	805	2072
LF FFT										
5	125	381	625	992	2304	58	274	523	937	2595
6	137	456	771	1254	3020	56	266	506	903	2483
7	109	407	718	1210	3072	58	273	519	924	2525
8	73	328	611	1071	2858	60	283	536	951	2581
9	58	322	630	1135	3084	62	292	552	978	2636
10	63	385	754	1345	3517	64	303	571	1008	2701
HF FFT										
5	111	383	675	1158	3171	14	306	622	1069	2383
6	161	603	1072	1827	4765	30	353	699	1194	2701
7	149	616	1105	1870	4668	52	426	820	1393	3196
8	120	558	1011	1700	4078	80	502	941	1590	3701
9	116	621	1135	1896	4385	112	568	1039	1745	4120
10	121	776	1433	2378	5321	145	628	1122	1873	4486

LFnu AR						
	5	29	44	52	60	76
	6	26	42	50	58	75
	7	24	41	49	58	75
	8	21	38	47	56	74
	9	19	37	46	55	74
	10	16	35	44	54	74
HFnu AR						
	5	14	27	34	42	62
	6	14	31	40	50	72
	7	14	33	43	53	76
	8	16	34	44	54	74
	9	19	36	46	57	74
	10	21	38	47	57	73
LF/HF AR						
	5	0.52	1.06	1.52	2.18	4.51
	6	0.38	0.86	1.29	1.92	4.31
	7	0.33	0.76	1.17	1.79	4.27
	8	0.29	0.70	1.08	1.67	4.04
	9	0.27	0.63	0.96	1.48	3.49
	10	0.29	0.64	0.94	1.40	3.09
LFnu FFT						
	5	20	37	45	54	73
	6	18	34	43	52	72
	7	16	32	41	50	71
	8	15	30	38	48	69
	9	13	27	36	46	68
	10	12	26	34	44	67
HFnu FFT						
	5	29	45	54	62	80
	6	29	48	57	65	83
	7	29	50	59	68	84
	8	30	52	62	70	86
	9	32	55	64	72	88
	10	34	56	65	74	89
LF/HF FFT						
	5	0.28	0.57	0.83	1.19	2.49
	6	0.24	0.51	0.76	1.12	2.43
	7	0.20	0.46	0.69	1.04	2.35
	8	0.18	0.41	0.62	0.94	2.14
	9	0.16	0.37	0.57	0.86	1.96
	10	0.14	0.34	0.52	0.80	1.84

AR= autoregression; FFT= fast Fourier transform; HF= high frequency spectral power; LF= low frequency spectral power; LF/HF= ratio of low frequency power to high frequency power; mRR= mean RR interval; nu= normalized units; pNN50= percentage of consecutive normal RRI differing more than 50 ms; RMSSD= root mean square of successive differences; SDNN= standard deviation of normal-to-normal intervals

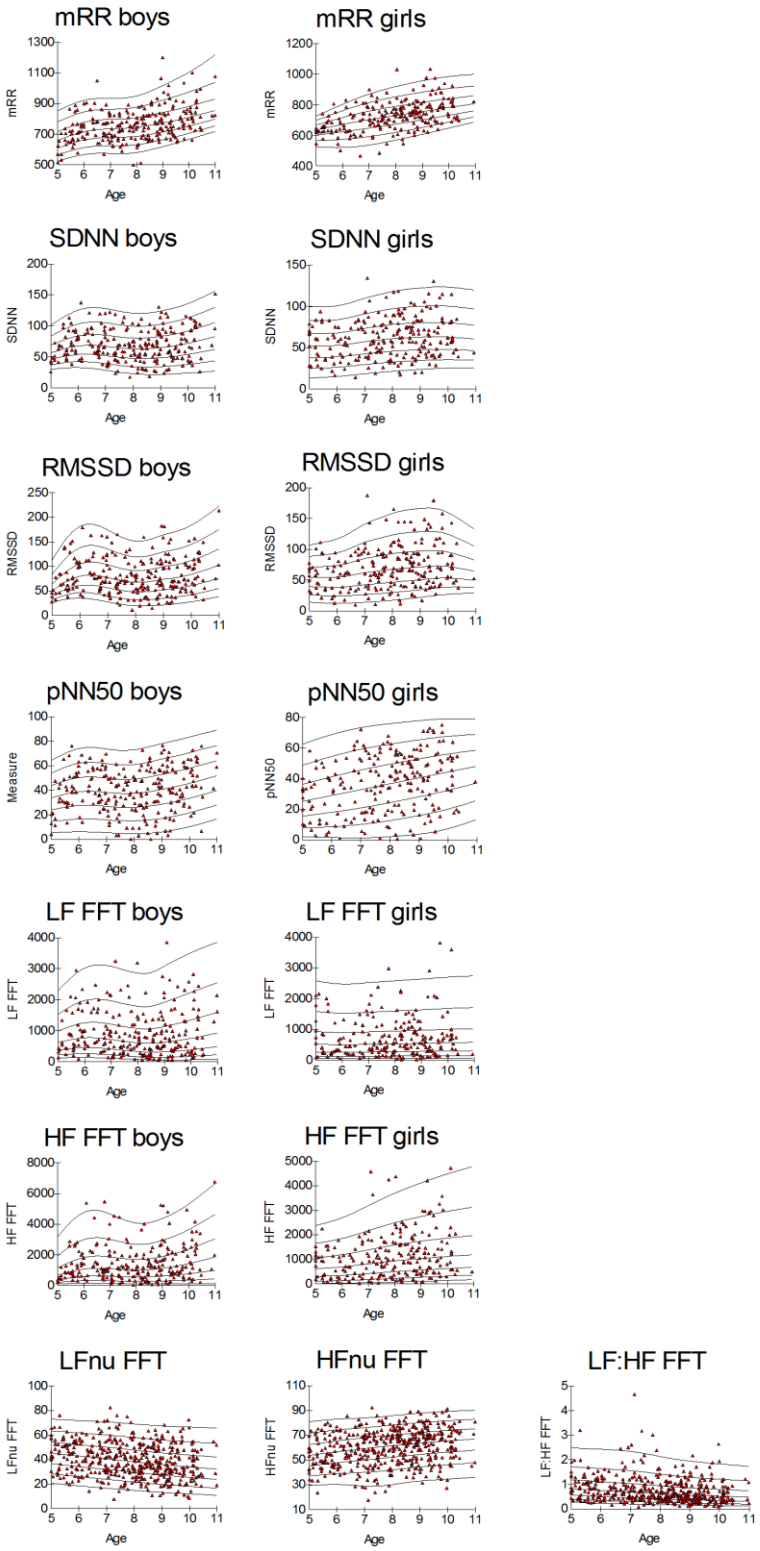

Figure 20: Age- and sex- specific percentile curves for HRV measurements in 460 children: 2.5, 10, 25, 50, 75, 90 and 97.5th percentile

FFT= fast Fourier transform; HF= high frequency spectral power; LF= low frequency spectral power; LF:HF= ratio of low frequency power to high frequency power; mRR= mean RR interval; nu= normalized units; pNN50= percentage of consecutive normal RRI differing more than 50 ms; RMSSD= root mean square of successive differences; SDNN= standard deviation of normal-to-normal intervals

Comparison of two different frequency domain methods

The frequency domain parameters retrieved by AR and FFT were mutually compared in Table 9. Despite the high correlations, significant differences were seen between AR and FFT for all parameters with overall lower FFT values in LFnu and LF/HF and higher in HF, LF and HFnu. Only LF power seemed interchangeable (ICC>0.75), although the confidence interval was too large to accept it. Table 9 also shows the limits of agreement of LFnu and HFnu as percentage (e.g. the AR LFnu was between -10% and 26% of the FFT value for 95% of the cases). For the log-transformed data, means and limits of agreement were expressed as ratio e.g. the AR LF will be between 0.4 and 1.4 times the FFT value. Higher differences were seen between FFT and AR with increasing power, more specifically there was more overestimation by the FFT method (because of the negative trend in Bland-Altman graph). For LFnu no trend was seen, but differences became smaller with increasing HFnu. For the ratio, more FFT underestimation was seen with increasing power (positive trend in Bland-Altman).

Table 9: Comparison of the two frequency domain models FFT and AR: correlation, paired t-test, bias, lower and upper limit of agreement, trend and intraclass correlation

	Correlation	Mean difference (AR-FFT); p-value	95% interval: lower and upper limit of agreement	Upwards or downwards trend[†]	ICC (95%-CI)
Log-transformed variables (mean and limits represent ratios)[$]					
LF (ms²)	0.94*	0.7**	0.34- 1.4	r= -0.69, p<0.001	0.87 (0.34-0.95)
HF (ms²)	0.97*	0.4**	0.2 - 0.7	r= -0.95, p<0.001	0.68 (0.03-0.91)
LF/HF	0.84*	1.7**	0.8 - 3.6	r=0.62, p<0.001	0.61 (0.08-0.85)
Non-transformed variables					
LFnu	0.79*	8.0**	-9.7% - 25.8%	No trend	0.68 (0.19-0.84)
HFnu	0.78*	-16.1**	-35.9% - 3.8%	r= 0.15, p=0.001	0.48 (0.95-0.78)

*AR= autoregression; CI= confidence interval; FFT= fast Fourier transform; HF= high frequency spectral power; ICC= intraclass correlation; LF= low frequency spectral power; LF/HF= ratio of low frequency power to high frequency power; nu= normalized units; *=p<.001; **=p<.001 for paired t-test; [†] trend in the Bland-Altman graph, [$] for these non-normally distributed data, log-transformed data were used for the analyses with an antilog retransformation afterwards, thus mean bias and limits of agreement represent ratios.*

Determinants of HRV parameters

The sex and age effects on HRV parameters were examined using two-way ANOVA. A sex*age interaction was found for SDNN, RMSSD, pNN50, AR HF and FFT HF, giving higher values for boys at the ages 5 and 6 and no sex differences at the older age. An age effect was seen for all variables except for LF (both AR and FFT). Detected sex differences were the same as for Table 7. When analysing the age effect with polynomial ANOVA, a cubic trend was seen for boys in all variables for which the sex*age interaction was significant. In girls, only linear trends were seen. Figure 21 shows these age and sex effects on mRR (example of the linear age effect) and RMSSD (example of the interaction effect).

Multiple regression analyses for age, time point and lifestyle factors (physical activity, physical fitness and body composition) were executed. Multiple regression results of physical fitness are shown separately in Table 10, as these were most significant and novel. Age was indeed correlated with both time and frequency domain parameters in both boys and girls, although more explicitly in girls (highest beta=0.474). The time point had only very minimal influence and this was restricted to boys. The model with age and time point could explain 17.6% and 13.9% of the variance in mRR for boys and girls respectively. R^2 change values ranged between 3.3% and 3.9% for physical activity, between 2.9% and 9.2% for physical fitness and between 1.6% and 4.9% for body composition. In boys, most physical fitness parameters and also physical activity were positive determinants, while body composition could barely serve as determinant (only one association with FFM). In girls, only FFM and arm strength showed a positive HRV association. Among physical fitness parameters, the sprint time had the largest effect. For body composition, only positive associations for FFM were seen, but no effect of FM, fat% and BMI was found. Determinant associations were in the same line for the AR and FFT method, although some differences were seen. After correction for body composition (FFM), all physical fitness and activity associations remained significant. Physical fitness even had a relevant impact over and above physical activity (after correction for MVPA).

After correction for mean HR, age remained significant (except for mRR). All significances for time point and body composition disappeared and only sprint time and physical activity remained significant in boys (data not shown).

Table 10:Multiple regression: physical fitness as determinant of HRV[$]

	Time domain				Frequency domain with AR					Frequency domain with FFT				
	mRR	SDNN	RMSSD	pNN50	LF	HF	LFnu	HFnu	LF/HF	LF	HF	LFnu	HFnu	LF/HF
Boys														
Arm strength	**0.20***	0.15	0.18	**0.22***	0.11	0.17	0.18	0.07	-0.12	0.09	0.16	-0.13	0.13	-0.11
Maximal jump	**0.22****	**0.22***	**0.23*†**	**0.26**†**	**0.21***	**0.23***	-0.06	0.05	-0.06	0.13	**0.22***	-0.14	0.14	-0.14
Sprint time	**-0.29****	**-0.37**†**	**-0.40**†**	**-0.36**†**	**-0.27****	**-0.38**†**	0.22	-0.19	**0.22***	-0.21	**-0.36**†**	**0.23***	**-0.23***	0.22
VO_{2max}	**0.17***	0.13	**0.17***	**0.20***	0.12	0.16	-0.03	0.11	-0.09	0.12	**0.16***	-0.07	0.07	-0.06
Girls														
Arm strength	**0.24*†**	0.21	0.19	0.20	0.13	**.239***	-0.19	0.16	-0.18	0.16	**0.23***	-0.08	0.08	-0.10
Maximal jump	0.09	-0.01	-0.02	0.07	-0.02	0.00	-0.06	-0.02	-0.02	0.02	0.02	0.00	0.00	0.00
Sprint time	-0.10	.-.129	-0.11	-0.19	-0.08	-0.12	0.12	-0.05	0.07	-0.12	-0.17	0.07	-0.07	0.06
VO_{2max}	0.02	0.11	0.10	0.10	0.14	0.04	0.10	-0.12	0.12	0.14	0.06	0.11	-0.11	-0.12

*AR= autoregression; FFT= fast Fourier transform; HF= high frequency spectral power; LF= low frequency spectral power; LF/HF= ratio of low frequency power to high frequency power; mRR= mean RR interval; nu= normalized units; pNN50= percentage of consecutive normal RRI differing more than 50 ms; RMSSD= root mean square of successive differences; SDNN= standard deviation of normal-to-normal intervals; * p<0.05; ** p<0.01; [†]= stayed significant after correction for physical activity (moderate-to-vigorous activity); [$] Standardized coefficients for the separate physical fitness parameters, corrected for age and time point*

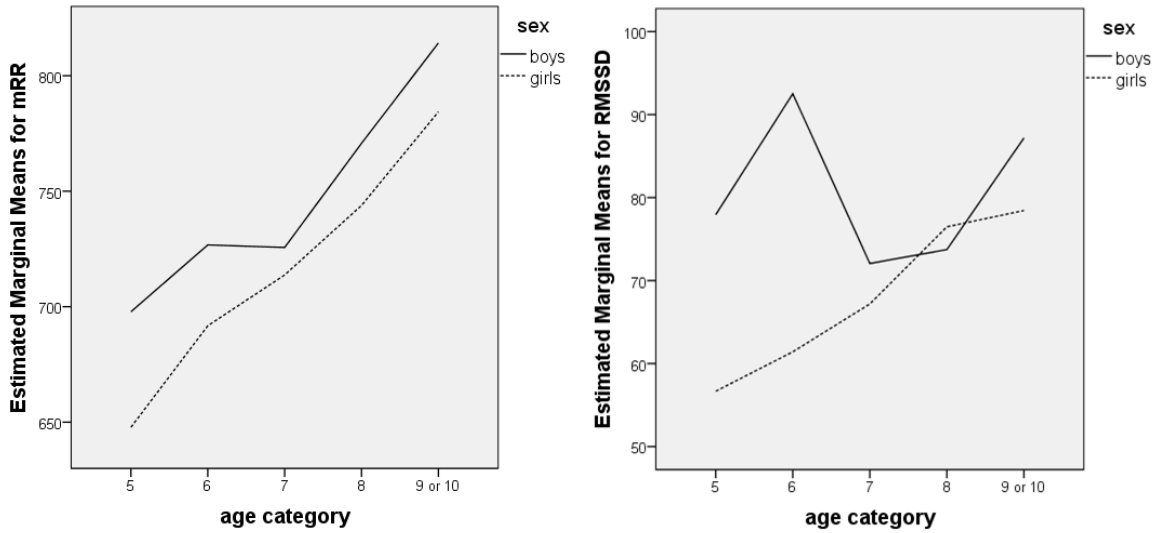

Figure 21: Age and sex effects: example on mRR (linear age effect) and RMSSD (age*sex interaction)

mRR= mean RR interval; RMSSD= root mean square of successive differences

Discussion

To our knowledge, this is the first study giving age- and sex-specific percentile values for an extensive battery of HRV parameters (both time and two frequency domain parameters) in a large sample of children and using the sophisticated LMS software. It is crucial as most of the children's HRV parameters were sex and age dependent and as the two frequency domain methods AR and FFT were not interchangeable. Moreover, physical fitness was a major positive determinant, especially in boys.

Descriptive statistics and percentile values

Our data indicate sex and age differences in HRV parameters in young children. Generally time and frequency domain parameters were higher in boys and increased with age. There were no sex differences for LFnu, HFnu and LF/HF and for the age-related decreases in LF/HF and LFnu. Because of the age and sex differences and an equal distribution of our population over age and sex, we reproduced age- and sex-specific percentile values. The LMS software had the advantage of allowing non-linear changes with age (Cole and Green 1992). It was indeed visible in the wave-like age-related year-to-year changes in boys, particularly in parameters with an age*sex interaction. When such a wave-like change was present, values decreased at age 7-8 and increased again afterwards. This pattern was never seen in girls. The wave-like change of LF in boys versus almost no increase in girls, could explain the absence of an age effect in the two-way ANOVA for LF. This highlights the importance of

considering sex-specific analyses and using non-linear percentile curves. Next to the general age-related increase, decreases were seen for LF/HF ratio and LFnu. Indeed, decreases in LF/HF could be caused by decreases in LFnu concordant with increases in HFnu. Furthermore, FFT and AR methods gave similar trends in age-related parameter changes but large differences in the classification between both methods exists (based on LF/HF ratio). The physiological meaning of these AR/FFT differences remains to be elucidated.

Comparison of our percentile values with those previously reported would be not straightforward because of methodological and population differences. Nonetheless, we tried to compare them with the large study of Galeev et al. giving age- and sex-specific short-term reference values in 6- to 16-year-old Russian children. They showed overall higher LF/HF values, higher LF values and slightly lower HF values than our study, but the time domain parameters (mRR, SDNN and RMSSD) showed the same trend as ours (Galeev, Igisheva et al. 2002). Also, our values very much resembled the reported RMSSD in German children (Longin, Dimitriadis et al. 2009) and LF and HF in Dutch children (Bosch, Riese et al. 2009).

Comparison of two different frequency domain methods

Frequency domain methods can be classified as nonparametric (FFT) and parametric (AR). FFT has the advantage of simple algorithms with high processing speed, while AR needs more time in choosing the AR model. This stimulates the frequent use of FFT. As FFT and AR were not interchangeable in our study, we could not recommend the exclusive use of FFT to cut down processing time. This AR-FFT non-interchangeability is also reflected by light dissimilarities in their association with determinants (Table 10). Our results are largely in agreement with studies in healthy adults (Fagard, Pardaens et al. 1998, Badilini, Maison-Blanche et al. 2000, Pichon, Roulaud et al. 2006): (1) AR and FFT values were significantly correlated for all measurements, although significant differences were seen, (2) the limits of agreement were wider for the ratio than for LF and HF separately, (3) the LF and HF power were overestimated with FFT, (4) the ratio was underestimated with FFT, and (5) a positive trend was seen for the ratio. Research in adults is more in disagreement on the AR/FFT differences for normalized units, but we have seen FFT underestimation in LFnu and FFT overestimation in HFnu. Overall, the limits of agreement were somewhat lower than those published in adults (Fagard, Pardaens et al. 1998). In a diabetic adult group, the methods were not interchangeable and the FFT was recommended as the preferred method as it has better reproducibility over days (Chemla, Young et al. 2005). Nevertheless, previous research has

also shown some agreement between the FFT and AR e.g. the circadian patterns and dynamic trends using the passive tilt test were equal (Badilini, Maison-Blanche et al. 1998, Badilini, Maison-Blanche et al. 2000).

Theoretically, the FFT-AR non-interchangeability may be explained by methodological differences in the preprocessing and in the power integration procedure used by the 2 spectral approaches (Badilini, Maison-Blanche et al. 2000): (1) In FFT preprocessing, the initial sequence is divided in multiple shorter overlapping sequences, each of them independently detrended by mean subtraction and as such trends are mostly removed by FFT. (2) Power is assessed with FFT using the integral (area under the curve) calculated from lower to upper band limit and with AR using the effective power associated with a spectral component with central frequency inside the band. As a result, the big tail of the larger component invades the band of the neighbour component and the corresponding power will be assigned to the other band by the FFT method, giving larger powers in FFT. The higher power in FFT was also seen in our population, with sometimes doubled values for FFT. In the reference paper of our used software, the output example also showed much higher powers in FFT (Niskanen, Tarvainen et al. 2004). The influence of these processing differences on interchangeability was even more highlighted as the FFT was comparable to AR in a previous research when the preprocessing and the power integration procedure was the same in AR and FFT (i.e. also using integration for AR) (Badilini, Maison-Blanche et al. 1998).

Determinants of HRV parameters

Our results confirm the sex differences as found in adults (Umetani, Singer et al. 1998) and adolescents (Faulkner, Hathaway et al. 2003) i.e. lower values in females. Nevertheless, no sex differences were seen in the sympathovagal balance (LF/HF), as was also shown in an adolescent study (Fukuba, Sato et al. 2009). Previous studies with young children as in our sample, showed only sex differences from 9 years onwards (Galeev, Igisheva et al. 2002) or not at all (Goto, Nagashima et al. 1997).

Age differences were already discussed in the percentile values section giving overall wave-like increasing trends (especially in boys) except for decreases in LFnu and LF/HF ratio without wave-like patterns. Most child studies found a HRV increase until 6 or 9 years old with a decrease or stagnation afterwards (Finley and Nugent 1995, Goto, Nagashima et al. 1997, Massin and vonBernuth 1997, Silvetti, Drago et al. 2001). Our findings confirm this

increasing trend although we also detected in boys the wave-like changes as found in a large sample of 6- to 16-year-old children (Galeev, Igisheva et al. 2002). The observed HRV age changes may reflect the development of vagal and sympathetic control of the heart with regulatory shifts. Indeed, neural autonomic functions mature over childhood attaining peak levels in adolescence with an improved cardiovagal autonomic function (Lenard, Studinger et al. 2004). Previously, increasing cholinergic and decreasing adrenergic modulation was seen over the first 14 years of child development, resulting in a decreasing LF/HF (Massin and vonBernuth 1997).

Circadian HRV rhythms in children exist with a midnight peak of time domain and LF and HF values and a trough for LF/HF (Massin, Maeyns et al. 2000). In our study, the time point (daytime) showed only a small influence on PA and only in boys, with lower values later on the day. If possible, recordings have to be done in a restricted time frame. Nevertheless, large-scale studies are not inevitably of lower quality by measuring over a larger time frame of the day.

Both decreased PA and increased SA are risk markers predicting morbidity (Thayer, Yamamoto et al. 2010). Consequently, we examined the influence of lifestyle related parameters such as physical activity, physical fitness and body composition on PA and SA.

Physical activity has been related to better HRV indices, especially a higher PA (Gutin, Howe et al. 2005, Krishnan, Jeffery et al. 2009), although sometimes only found with vigorous activity (Buchheit, Platat et al. 2007). Furthermore, physical training has improved HRV in children with low baseline HRV (Nagai and Moritani 2004) (increased LF and HF). In our study, higher physical activity was associated with higher PA (RMSSD, pNN50 and HF) and consequently higher values in the dually innervated LF.

Apart from physical activity, also physical fitness should be considered. After all, only low-to-moderate correlations have been detected between children's objectively measured activity and fitness (Dencker and Andersen 2011) and individual differences exist in the response to physical activity (Bouchard and Rankinen 2001). While activity is a lifestyle factor, physical fitness is a physiological condition and could as such be more related to physiological markers as HRV, as we have seen. Previously, physical fitness was also shown to be better related to cardiovascular risk factors than physical activity in children (Hurtig-Wennlof, Ruiz et al. 2007). HRV associations with physical fitness have rarely been published and were limited to VO_{2max} measurement. Higher RMSSD had previously been found in more fit

children (Gutin, Howe et al. 2005), while no effect of fitness was seen in an adolescent group (Brunetto, Roseguini et al. 2005). We observed beneficial effects: higher mRR and PA (HF, RMSSD, pNN50) with increasing fitness and with the sprint time as most influencing determinant. In girls, arm strength was the only determinant. Taken together, quite similar associations were seen for physical fitness and physical activity. Nevertheless, physical fitness was a more powerful determinant because of the abundant and higher coefficients that remained significant even after correction for physical activity.

We tested the effect of body composition in our low-overweight population (7%), but no effect of fatness was seen. Beneficial associations of HRV variables for FFM (the counterpart of fat) were mainly present in girls and FFM-mRR was the only association remaining significant after correction for physical fitness. As HRV associations of physical fitness and activity remained significant after correction for body composition and as FFM could be causally linked to physical activity, the fitness determinants might be more potent. There is ample evidence from literature that HRV differs between obese and lean children (Gutin, Howe et al. 2005, Kaufman, Kaiser et al. 2007) with an overall lower PA, independent of physical activity (Nagai and Moritani 2004). Changes in SA were more likely to depend on obesity duration (Rabbia, Silke et al. 2003). This could explain the lack of associations in a low-overweight population. In another low-to-moderate-overweight sample, no differences were seen except for lower HR with higher FFM in girls (Krishnan, Jeffery et al. 2009).

Apart from sex differences in the HRV parameters, sex differences were also seen in the associations with determinants. Previously, similar sex differences were seen with physical activity associations in boys only and FFM associations in girls (Krishnan, Jeffery et al. 2009). These sex differences could perhaps be partially explained by our population characteristics: a higher prevalence of overweight in girls and higher fitness in boys. The sexes were equally distributed over age and time point, so this could not explain the sex differences in the associations. Moreover, the physical fitness tests were previously shown reliable and no sex difference in reliability exists (Ortega, Artero et al. 2008).

Strengths and limitations

Standardized HRV measurements with an extra manual quality control were executed in a large healthy child population of 460 subjects. Next to the frequently used and quick processing FFT method, we have also implemented the more elaborated AR method. Therefore, these two methods could be compared in children for the first time. The effort of

model selection for AR in combination with a manual quality control has rarely been done in such a large population and to the authors' knowledge never before in children. It initiated the opportunity to represent percentile values. Current published reference values for children have often been based on smaller samples (Finley and Nugent 1995, Goto, Nagashima et al. 1997, Massin and vonBernuth 1997, Faulkner, Hathaway et al. 2003, Longin, Dimitriadis et al. 2009), except for the large Russian study of Galeev et al. (Galeev, Igisheva et al. 2002). The LMS method allowed for non-linear HRV changes with age. It was a major strength as wave-like changes were indeed seen in boys.

Next to age and sex differences, we also examined lifestyle factors. Therefore, standardized techniques have been implemented: objectively measured physical activity instead of the frequently used subjective questionnaires, a well-known physical fitness test battery with proven reliability and body fat percentage measured with specialized technology. Examining physical fitness was a major asset as it has rarely been studied in relation to HRV and as it was a more potent determinant than physical activity in our study. Furthermore, physical fitness and activity were related to HRV independent of other determinants and HR.

Nevertheless, some limitations are connected to these determinants. (1) We had only a low percentage of overweight in our sample (7%). Although we found no influence of body fatness on HRV, there might be an influence in high-overweight populations. (2) Another checked determinant was the time point of registration, with almost no influence on HRV. To ascertain this statement, future studies should measure the same child on several different time points to examine the influence of time more directly. Alternatively, we could recommend 24h HRV recordings if this is logistical feasible as these take into account the diurnal rhythm and the other spectral components 'very low frequency' and 'ultra low frequency'. (3) Finally two measurement limitations related to the used software should be considered. (3a) Non-stationarity was only manually inspected (no outliers, an equidistance, minimal variation, stable mean and Gaussians distributions) and this might not detect all non stationarities, increasing the likelihood to overestimate the contribution of the sympathetic activity (Magagnin, Bassani et al. 2011). (3b) Although visual respiratory observation was done during the registration, breathing rate was not measured with an extra channel, possibly confounding our linear methods. Breathing rate was shown to be negatively related with LF and HF power, but had no influence on time domain parameters (Brown, Beightol et al. 1993, Penttila, Helminen et al. 2001). Nonlinearities are more likely to happen with a low breathing rate (e.g. at 10/min breathing rate), a long RRI and in the presence of important respiratory

sinus arrhythmia (Porta, Baselli et al. 2000). Nevertheless, the influence of nonlinearities will be minimal in our population as children have a general high breathing rate (18-21/min mean in our age range) (Wallis, Healy et al. 2005) and short RR-interval; and respiratory sinus arrhythmia induced by the Valsalva manoeuvre was more likely abolished.

Conclusions

Performing short-term HRV measurements with a polar chest belt was easily feasible as part of a large epidemiological study in children. Our sex- and age-specific percentile values can be used in the future and highlighted the importance of considering sex differences and using non-linear percentile curves. Overall, girls had lower HRV and age-related parasympathetic increases and sympathetic decreases were seen. Apart from physical activity and fat-free mass, in particular fitness (sprint time) influenced HRV in a favourable way by increased parasympathetic activity, especially in boys and independent of body composition, physical activity and heart rate. Consequently, future HRV studies in children should consider age, sex and physical fitness. Furthermore, our study showed that the quick and frequently used FFT method was not interchangeable with AR.

3.

Negative life events, emotions and psychological difficulties as determinants of salivary cortisol in Belgian primary school children.

Michels N, Sioen I, Huybrechts I, Bammann K, Vanaelst B, De Vriendt T, Iacoviello L, Konstabel K, Ahrens W, De Henauw S

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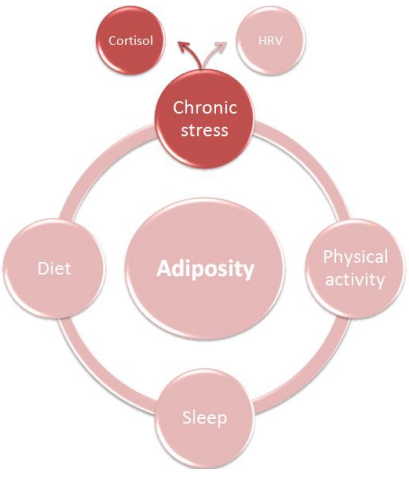

It is not stress that kills us,

it is our reaction to it.

(Hans Selye)

ABSTRACT

PURPOSE: This paper describes whether children's life events, emotions and psychological problems are related to their salivary cortisol patterns and whether this is different between sexes.

METHODS: In 385 children (5 to 10 years old) participating in the ChiBS study, salivary cortisol samples were collected when waking up, 30 minutes and 60 minutes after wake up and in the evening on two consecutive weekdays. Moreover, data on children's life events, emotions and problems were collected. Statistical analysis was done separately for boys and girls by multilevel growth curve modelling with adjustments for age, body mass index, socio-economic status and wake up time.

RESULTS: In boys and girls with more negative life events during the last 3 months, the diurnal cortisol slope was steeper (more decline). Boys with higher self-reported happiness showed lower overall, morning and evening cortisol levels. In contrast, the diurnal slope was steeper (more decline) in boys with emotional problems due to higher morning values. In girls, peer problems were associated with lower overall and morning cortisol levels.

CONCLUSIONS: Children's salivary cortisol patterns were related to some negative life events, emotions and problems, although differently in boys and girls. As such, sex-differences in HPA functioning are already present in young children. Most findings support the upregulation of the cortisol response to stress, although lower morning values were found in the presence of peer problems in girls. Future studies should focus on sex differences, positive emotions and the diurnal cortisol slope.

Introduction

Problem behaviour and stress are frequent phenomena in children, with prevalence estimates between 5 and 26% (Brauner and Stephens 2006). Stress is linked not only to psychological but also physiological health complaints through behavioural responses and, more importantly, changes in the neuroendocrine system (Cohen, Janicki-Deverts et al. 2007). One of the major neuroendocrine systems adapting the organisms to stress situations, is the hypothalamus-pituitary-adrenal (HPA) axis with cortisol as hormonal end product.

Cortisol secretion has a circadian rhythm with lowest levels in the first half of the night and a peak in the early morning. Apart from this circadian rhythm, there is also a cortisol awakening response (CAR) showing a quick cortisol increase within 30 minutes after wake up (Fries, Dettenborn et al. 2009). Salivary cortisol is a reliable reflection of serum free cortisol and due to its non-invasive character, it is routinely used as a stress biomarker in children (Tornhage 2009). These cortisol patterns can be influenced by stress exposure, showing the psycho-physiological pathway through which psychosocial factors could influence health (Cohen, Janicki-Deverts et al. 2007). As such, research on the associations between psychometric data and cortisol patterns in children can help in disentangling the HPA functioning, in identifying psychobiological developmental pathways and in the prevention of stress-induced pathology.

Negative life events have been associated with both emotional and behavioural symptoms in children (Grant, Compas et al. 2004, Timmermans, van Lier et al. 2010) and children exposed to a moderate degree of this psychosocial load, differed in their cortisol levels (Gustafsson, Gustafsson et al. 2006). Stress has often been associated with higher cortisol values (Selye 1956, Michaud, Matheson et al. 2008). Nevertheless, the associations are quite complex, giving conflicting results and a hyper-/hypo-cortisolism hypothesis was published suggesting that recent exposure to a stressor may initially elevate cortisol levels, while the HPA axis may develop a counter-regulatory response of cortisol lowering after extended exposure to stress (Heim, Ehlert et al. 2000). This hypocortisolism is an allostatic situation characterised by lower morning cortisol, higher evening cortisol and a flatter diurnal slope and may also be a common phenomenon in children (Gunnar and Vazquez 2001). Apart from this, variability in cortisol response is attributable to the nature of the stressor (social or physical) and also to the person facing it (emotional response and psychiatric sequelae) (Miller, Chen et al. 2007).

Furthermore, age and sex differences are observed in the HPA development. In adolescents, nonlinear age related increases were seen for both basal cortisol and cortisol reactivity and higher basal cortisol and cortisol reactivity were seen in pubertal girls compared to boys (Netherton, Goodyer et al. 2004, Gunnar, Wewerka et al. 2009). Most of the available research has been done on pubertal children and only few data are available in young children. Nevertheless, puberty-related increases in stress response and emotional reactivity have been reported (Spear 2009). It is still unclear whether sex differences also exist in young children in which pubertal hormonal pathways are not yet activated. Possibly, the demonstrated sex differences in psychological functioning and development (Crick and Zahn-Waxler 2003) could explain sex differences in cortisol reactivity.

To fill this research gap, we investigated the response of cortisol patterns on challenges during children's development, in a non-clinical, healthy population of 5-10 year old children. This was done by studying the relationship of healthy children's negative life events, emotions and problems with their salivary cortisol patterns (overall elevation, diurnal slope and CAR). Special focus is dedicated to sex differences in these preadolescent children. An advanced multilevel model enabled a detailed analysis of cortisol patterns allowing to simultaneously assess all parameters related to overall concentrations as well as CAR and diurnal slope (Hruschka, Kohrt et al. 2005, Adam 2006). Moreover, apart from several stress-inducing psychometric data (negative events, emotions and problems), also the role of positive emotions is considered, which is new for this young age group.

Methods

Participants and general procedures

Participating children were part of the Belgian baseline ChiBS study. Detailed aims, design, methods, population and participation characteristics can be found in chapter 2 "Methodology".

Although 444 children provided salivary cortisol samples, only 385 children were included for statistical analysis because of uncompleted questionnaire data. No differences in psychometric, salivary cortisol or socio-demographic data were observed between the included and excluded children.

Questionnaires on life events, emotions and problems

Life events (child-reported)

The 'Coddington Life Events Scale' for children (CLES-C) (Coddington 1972), a tool for clinicians, was used to identify potential stress-inducing events. The English questionnaire was professionally translated into Dutch and translated back into English. This validated 36-item questionnaire assesses the frequency and timing of events relevant for this age group during the last year and it results in a *'life change units'* score for different time spans (0-3 months ago, 0- 6 months ago, 0-9 months ago and 0-12 months ago). Apart from a total event (negative and neutral/positive events) score, also a score for negative events was calculated following the questionnaire guidelines.

Emotions (child-reported)

Children were inquired for their recent feelings of anger, anxiety, sadness and happiness as reported in chapter 2 "Methodology".

Problems (parent-reported)

The parents were asked to complete the 'Strengths and Difficulties Questionnaire' (SDQ), a self-administered questionnaire on their children's behavioural problems over the last 6 months (Goodman 1997). A *'problems'* score was calculated by summing up the subscales for emotional symptoms, conduct problems and peer problems, in accordance to the questionnaire manual. The prosocial scale was considered as a separate concept namely as a strength.

Salivary cortisol

Saliva was collected at home via Salivette synthetic swabs (Sarstedt, Germany) immediately after wake up (T0), 30 minutes after wake up (T30), 60 minutes after wake up (T60) and in the evening between 7 and 9 PM (Tev). More details on sampling and analyses can be found in chapter 2 "Methodology".

Confounding variables

Based on previous research on cortisol values, age, socioeconomic status, wake up time and body mass index (BMI) z-score were considered as potential confounding variables (Adam

and Kumari 2009). Parental education (as marker for socioeconomic status) and children's BMI was collected as described in chapter 2 "Methodology". Day-specific wake up time was reported in the parental checklist for saliva sampling. Children's age was calculated from birth date and examination date.

Statistical analyses

Due to a skewed distribution, cortisol concentrations were transformed by the natural logarithm. Mann-Whitney U test showed sex differences in psychometric data and sex differences were also observed in the exploratory correlation analyses. Therefore, the statistical model was built for girls and boys separately. Hierarchical linear modelling (HLM) was used to analyse the relation between the children's cortisol pattern and questionnaire data (life events, emotions and problems), adjusted for potential confounders (age, BMI, parental education and wake up time). HLM is a variant of multiple linear regression useful for data with a nested design, which is the case in this study as repeated cortisol measurements were obtained for each participating child. Therefore, a two-level model on the dependent variable 'cortisol' was used with the intra-individual parameters modelled at level 1 (cortisol pattern created by time of day) and the inter-individual parameters (questionnaire data and the personal characteristics as possible confounders) at level 2. HLM is increasingly used and has its advantages when analysing cortisol data: a high tolerance for within- and between-subject variation in sampling time, simultaneous modelling of multiple cortisol parameters (elevation, diurnal slope and cortisol awakening response) and added statistical power because of the within-person repeated-measures design (Hruschka, Kohrt et al. 2005). The HLM was performed in the HLM/2L program (version 7.0), using an approach similar to the one published by Adam (Adam 2006). The significance level was set to $p<0.05$.

At level 1, the child's cortisol values were predicted by the time of day, to estimate the shape of each child's cortisol curve during the day. Time of day values were expressed as 'number of hours since wake up' for each participant each day and centred to midday as six hours post-awakening. A curvilinear model did not fit better than the linear model. To characterise the CAR, a design variable was created assigning the value 1 to the sample taken 30 minutes after wake up, and the value 0 to all other samples. By representing the CAR as a separate variable in the model, the CAR became a separate coefficient that could be predicted independently of the other parameters, such as the diurnal slope. A dummy variable for day of measurement was included to account for possible systematic cortisol differences across days. The reported

day-specific wake up time, considered as a cortisol-confounder, was also included as a level 1 parameter because of its day-dependence.

In an exploratory analysis, each relevant parameter resulting from the questionnaires (total and negative events 0-3, 0-6, 0-9 and 0-12 months ago; the emotions happy, anger, anxiety, and sadness; and the SDQ-scores) was entered as a level 2 predictor of each of the relevant level 1 predictors (intercept, slope and CAR) and these models were adjusted for potential confounders (age, BMI, parental education and wake up time). The significant level 2 predictors were then entered simultaneously (all remained significant) to result in one model although also the other level 2 predictors were retested on their significance in this model. No multicollinearity was detected in the final model using the tau-matrix. Post-hoc HLM analyses were performed to examine the effect of the significant level 2 predictors on morning and evening cortisol by entering a non-centred 'time since wake up' or by centring at 12 hours post-awakening, respectively.

Maximal effects of the psychosocial factors on cortisol concentrations in our population can be calculated as follows: (% difference/scale point, as displayed in Table 12 or Table 13) * (range of this scale in the sample, as displayed in Table 11). Nevertheless, these predictions should be interpreted with caution as they may be influenced by outliers.

Results

In total, 183 boys and 202 girls were included in this study. Descriptive data resulting from the questionnaires, salivary cortisol samples and possible confounding variables are provided in Table 11. No significant differences between boys and girls were observed for salivary cortisol and the sociodemographic characteristics, while anxiety ($p=0.015$), sadness ($p=0.001$) and prosocial behaviour ($p=0.004$) were higher in girls and conduct problems ($p<0.001$) were higher in boys. When using the multilevel model not stratified for sex, no sex difference was seen in overall mean cortisol, although girls showed somewhat steeper diurnal slope ($p=0.30$, 0.9% higher).

Table 11: The children's personal and sociodemographic characteristics and descriptive data from the cortisol values and questionnaires on life events, emotions and problems

	Boys (n=183)				Girls (n=202)			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Salivary cortisol (nmol/L)								
Immediately after wake up	12.66	6.12	1.66	56.00	13.84	8.96	1.49	76.41
30 minutes after wake up	13.28	8.17	2.07	77.79	14.61	6.96	2.01	55.72
60 minutes after wake up	8.97	5.00	0.74	40.55	9.15	5.98	1.27	44.14
Evening	2.17	1.97	0.52	17.93	2.11	1.82	0.50	14.65
Personal and sociodemographic characteristics								
Age (years)	8.44	1.18	6.21	10.99	8.39	1.20	5.23	10.93
Average wake up time (h; decimal)	6.97	0.45	5.95	8.50	6.95	0.52	5.83	8.28
Body mass index (z-score)	-0.36	1.74	-2.57	2.68	-0.18	1.23	-3.66	4.10
Highest parental occupation (ISCED-level between 0-5)	4.31	0.82	3.00	5.00	4.14	1.00	1.00	5.00
Child psychometric report								
Coddington Life Events Scale								
Total score last 3 months	50.07	61.63	0	357	44.67	55.66	0	265
Total score last 6 months	61.52	67.04	0	428	55.33	64.59	0	291
Total score last 9 months	68.01	69.52	0	447	65.39	68.04	0	343
Total score last 12 months	81.83	74.04	0	460	81.08	68.46	0	369
Negative event score last 3 months	27.14	43.76	0	220	28.64	41.00	0	172
Negative event score last 6 months	34.01	47.93	0	284	36.07	48.54	0	276
Negative event score last 9 months	37.63	50.36	0	303	42.11	52.29	0	328
Negative event score last 12 months	47.68	53.66	0	303	53.26	54.40	0	354
Emotions								
Happy	7.91	1.94	1	10	7.92	1.84	2	10
Angry	2.93	2.45	0	10	2.88	2.40	0	10
Sad	2.36	2.51	0	10	3.06	2.74	0	10
Anxious	1.80	2.64	0	10	2.36	2.85	0	10
Parental psychometric report of child								
Child's Strengths and Difficulties								
Total problems	5.08	3.55	0	18	4.81	3.51	0	19
Conduct problems scale	1.49	1.24	0	5	1.02	1.06	0	5
Emotional symptoms scale	2.28	1.94	0	9	2.57	2.03	0	9
Peer problems scale	1.36	1.49	0	7	1.27	1.49	0	7
Prosocial behaviour = strength	6.46	1.45	1	8	6.89	1.27	1	8

Based on the intra-class correlation, the between-subject variance in boys determined 32.8% of the total cortisol variance, while their within-subject variance across time was 67.2%. In girls, the between- and within-subject variance were 38.7% and 61.3%, respectively. Boys and girls showed the expected diurnal slope, with higher levels in the morning and an average decline of 15.8% per hour for boys and 15.6% per hour for girls. A CAR increase of 21.3% and 26.1% was seen, for boys and girls respectively. Wake up time was significant in both boys and girls, with a maximal 28.56% and 48.95% cortisol change, respectively.

Table 12 and Table 13 provide the final hierarchical linear model results for boys and girls, respectively. For level 2, only variables with significant effects were retained.

In boys, overall cortisol values were lower among those with higher levels of self-reported happiness, even after adjusting for negative emotions. Diurnal cortisol slope was steeper in boys having a high negative event score in the last 3 months and having a high emotional symptom score or total score based on the SDQ. Levels of anger, anxiety, sadness, other SDQ subscales, total event scores and negative event scores on longer time-period were not significantly associated with any of the cortisol pattern parameters. Remarkably, also the total SDQ problems score was significant, although to a lesser extent than the emotional subscale. Post-hoc analyses in Table 11 showed lower morning and evening cortisol levels in boys with a high score on happiness and higher morning cortisol levels in boys with a high emotional symptoms score.

In girls, overall cortisol values were negatively associated with peer problems and the diurnal cortisol slope was steeper when having a high negative event score in the last 3 months. Levels of happiness, anger, anxiety, sadness, other SDQ subscales, total event scores and negative event scores on longer time-period were not significantly associated with any of the cortisol pattern parameters. Post-hoc analyses in Table 14 showed lower cortisol morning levels in girls with peer problems.

The maximal cortisol difference between lowest and highest psychometric score was 2.2% in boys and 1.7% in girls for negative life events during the last 3 months, 3.6% for emotional symptoms in boys, 25.2% for happiness in boys and 26.6% for peer problems in girls.

Table 12: Hierarchical linear model showing association between children's cortisol and questionnaire data on life events, emotions and problems corrected for relevant confounders in boys (n=183)

Fixed effect	Coefficient	SE	t-value	df	p	Interpretation
Cortisol intercept	2.2680	0.3232	7.02	178	<0.001	
Happy	*-0.0277*	*0.0128*	*-2.16*	*178*	*0.032*	*-2.8%/scale point*
Time since wake up (= slope)	-0.1469	0.0027	-53.33	177	<0.001	-15.8%/hour
Negative events last 3 months	*-0.0001*	*0.0001*	*-2.01*	*177*	*0.045*	*-0.01%/scale point*
SDQ Emotional symptoms[(a)]	*-0.0037*	*0.0013*	*-2.74*	*177*	*0.007*	*-0.4%/scale point*
Cortisol awakening response	0.1935	0.0190	10.16	179	<0.001	+21.3% on T_{30}
Wake up time	-0.1065	0.0511	-2.08	182	0.039	-11.2%/hour
Day	0.0488	0.0281	1.74	182	0.084	Not significant

Note. Natural logarithmic transformed cortisol values were used as dependent variable. Time since wake up (centred as six hours post-awakening), cortisol awakening response dummy (value 1 for sample 30 minutes after wake up), day of testing dummy and wake up time were entered as level 1 variable. SDQ (strengths and difficulties questionnaire) and its subscales, child reported emotions (happy, angry, sad, anxious), total and negative life event scores (score for 0-3, 0-6, 0-9 and 0-12 months ago) and day-independent confounders (BMI z-score, age, parental education) were entered as level 2 predictor on each level 1 predictor. Variables in the final model represent the independent effect of each variable as all significant variables were entered simultaneously. Since cortisol values were log-transformed, the following transformation has been applied to the B coefficient for interpretation: B%change=[exp(B)]-1.

Interpretation issue. Since the diurnal slope is negative, decreases in slope result in a steeper decline (=higher slope).

[(a)] Also the total SDQ score was significant (β=-0.0014; p=0.043). This data was not included in the same final model for reasons of multicollinearity.

Table 13: Hierarchical linear model showing association between children's cortisol and questionnaire data on life events, emotions and problems corrected for relevant confounders in girls (n=202)

Fixed effect	Coefficient	SE	t-value	Df	p	Interpretation
Cortisol intercept	2.4114	0.4056	5.95	197	<0.001	
SDQ Peer problems	*-0.0389*	*0.0178*	*-2.19*	*197*	*0.030*	*-3.8%/scale point*
Time since wake up (= slope)	-0.1457	0.0031	-46.70	197	<0.001	-15.6%/hour
Negative events last 3 months	*-0.0001*	*0.0001*	*-1.98*	*197*	*0.048*	*-0.01%/scale point*
Cortisol awakening response	0.2320	0.0247	9.38	198	<0.001	+26.1% on T_{30}
Wake up time	-0.1966	0.0557	-3.53	201	0.002	-17.8%/hour
Day	0.0301	0.0367	0.82	201	0.414	Not significant

Note. Natural logarithmic transformed cortisol values were used as dependent variable. Time since wake up (centred as six hours post-awakening), cortisol awakening response dummy (value 1 for sample 30 minutes after wake up), day of testing dummy and wake up time were entered as level 1 variable. SDQ (strengths and difficulties questionnaire) and its subscales, child reported emotions (happy, angry, sad, anxious), total and negative life event scores (score for 0-3, 0-6, 0-9 and 0-12 months ago) and day-independent confounders (BMI z-score, age, parental education) were entered as level 2 predictor on each level 1 predictor. Variables in the final model represent the independent effect of each variable as all significant variables were entered simultaneously. Since cortisol values were log-transformed, the following transformation has been applied to the B coefficient for interpretation: B%change=[exp(B)]-1.

Interpretation issue. Since the diurnal slope is negative, decreases in slope result in a steeper decline (=higher slope).

Table 14: Post-hoc analyses for the significant level 2 predictors stratified by sex: effect on morning (wake up) and evening cortisol

	Morning[(a)]			Evening[(b)]		
	Coefficient	p	Interpretation	Coefficient	p	Interpretation
Boys						
Happy	-0.0209	0.030	-2.1%/scale point	-0.0331	0.024	- 3.4%/scale point
Negative events last 3 months	0.0001	0.302	Not significant	0.0002	0.731	Not significant
SDQ Emotional symptoms	0.0017	0.038	+0.2%/scale point	-0.0092	0.503	Not significant
Girls						
SDQ Peer problems	-0.0428	0.035	-4.2/scale point	-0.0130	0.452	Not significant
Negative events last 3 months	-0.0001	0.063	Not significant	-0.0001	0.620	Not significant

Note Since cortisol values were log-transformed, the following transformation has been applied to the B coefficient for interpretation: B%change=[exp(B)]-1. The level 2 predictors were entered on their own. SDQ= strengths and difficulties questionnaire

(a) Time not centred= wake up time

(b) Time centred at 12 hours post-awakening

Discussion

The salivary cortisol concentrations from this child population showed a clearly significant diurnal pattern with higher morning and lower evening values and a less clear, although significant, CAR. The diurnal slope was comparable to a similar child study (Adam 2006), but the CAR was quite low as not all children showed a morning increase. This was recently described in detail (Michels, Sioen et al. 2012). The higher within-person compared to between-person variability clearly illustrated the impact of collection time resulting in a broad diurnal pattern. Age was a significant and powerful determinant for cortisol in girls, while BMI and wake up time were significant in both boys and girls. Several associations of negative events, emotions and problems were observed, with the strongest for happiness in boys (21.6% cortisol difference between lowest and highest happiness) and for peer problems in girls (22.4% cortisol difference between lowest and highest peer problems) (see methods for formula).

Overall sex differences

The association of life events, problems and emotions with salivary cortisol found in this study was different in boys and girls. Indeed, sex-specific stress-cortisol relations have been previously published but are conflicting as e.g. a newly validated questionnaire on symptoms, emotions, events and coping found positive correlations with cortisol only in girls (Osika, Friberg et al. 2007), whereas in another study cortisol values were positively correlated with distress only in boys (Vigil, Geary et al. 2010). Almost all of these sex-specific associations were performed in children starting from 10 years old or older and as such covering adolescence. Still, a study on 5-year olds showed sex differences in the association with psychometric data giving significances of hyperactivity and emotional problems in boys and of positive emotions in girls (Hatzinger, Brand et al. 2007).

Several aspects could explain these sex differences in cortisol associations. First of all, sex differences on the HPA-axis itself have been published (Netherton, Goodyer et al. 2004, Gunnar, Wewerka et al. 2009). The literature overall indicates higher basal and stress-challenged cortisol in girls (Netherton, Goodyer et al. 2004, Rosmalen, Oldehinkel et al. 2005, Hatzinger, Brand et al. 2007, Gunnar, Wewerka et al. 2009), although this was not always the case in preadolescent children (Netherton, Goodyer et al. 2004, Gunnar, Wewerka et al. 2009). In our study, no overall cortisol sex differences and only a slightly steeper diurnal

slope in girls were detected. Secondly, differences in boys and girls have regularly been shown in the prevalence, developmental pathways and manifestation of psychopathology (Crick and Zahn-Waxler 2003) and in handling stressful situations (Hampel and Petermann 2005). After all, girls are considered more mature, have more advanced language skills and show more empathic and prosocial responses than boys during childhood (Crick and Zahn-Waxler 2003). In addition, gender could be a moderator in the relation between symptoms and subjective well-being (Derdikman-Eiron, Indredavik et al. 2011). So, the sex differences present in the psychometric data of this study are in line with general literature: more sadness, anxiety and prosocial behaviour in girls, while more conduct problems in boys (Crick and Zahn-Waxler 2003). In the next paragraphs, these sex differences are examined further in our group of young children and in relation to the specific psychosocial factors.

Associations between life events (CLES) and cortisol patterns

Concerning life events, no associations with cortisol were observed for total event scores but the diurnal slope was steeper for boys and girls when more negative events occurred during the last 3 months. This indicates that stress researchers should also consider the negative event score separately and not only the total event score that includes also some neutral/positive events. A recent study using the CLES in 9 to 16 year old children also found no association with wake up and evening cortisol, although the steepness of the diurnal slope was depending on the amount of lifetime adversities with a steeper slope in children having a moderate amount of adversities and a less steeper slope in children with a high amount of adversities (Gustafsson, Anckarsater et al. 2010). A possible explanation of this different result, is the time frame in which the events took place. Our observed steeper slope for children with more negative events might be explained by only considering events that took place during the last year. In contrast, the study of Gustafsson used lifetime adversities and suggested that the accumulation of adversities over time might be a trigger to develop the counter-regulatory hypocortisolism. Overall, the calculated cortisol difference in the presence of negative life events was only minimal. Furthermore, the questionnaire only measured the occurrence of events and not the subjective stress perception or coping. So, it is not known whether the reported events were perceived as stressful and how the children dealt with it (Miller, Chen et al. 2007). Consequently, this could have disturbed the ability to observe stronger associations.

Associations between behavioural problems (SDQ) and cortisol patterns

The impact of SDQ problems has already been related with increased evening cortisol (Gustaffson 2006). Considering the behavioural problems, total score, the emotional scale and the peer problem scale were related to cortisol. No significance of conduct problems was seen, which was in line with previous research in 10 to 12 year olds (Sondeijker, Ferdinand et al. 2007) and in 5 year olds using the same questionnaire (Hatzinger, Brand et al. 2007).

For boys, emotional symptoms and total SDQ score were associated with a higher slope and higher morning cortisol. This is quite in line with the study of Hatzinger et al. (2007) demonstrating a positive association between emotional symptoms and mean morning cortisol in boys, although they did not examine the diurnal slope. Indeed, a positive association between children's internalizing/emotional problems and morning cortisol was also seen in another cross-sectional analysis, while the longitudinal analyses showed negative associations (Ruttle, Shirtcliff et al. 2011), possibly reflecting the hyper/hypo-cortisolism hypothesis. In our study and in line with a previous publication (Crick and Zahn-Waxler 2003), the lower parental report of emotional problems was more frequent in girls, which could possibly be explained by the sometimes undisclosed feelings in girls and by the differential treatment of sons and daughters by the parents. This underreporting might impede significance in the associations of emotional problems reported by the parents with cortisol in girls.

We detected an opposite direction for peer problems: they were associated with lower cortisol in girls. As far as we know, no other studies have demonstrated this sex difference in our age group. Previously, lower cortisol was observed to be related to lower peer status at school in 15-year olds (West, Sweeting et al. 2010), although a study in preschoolers showed highest median cortisol in the least liked/most disliked children (Gunnar, Sebanc et al. 2003). Sex differences in children's peer context has been reviewed with girls being more sensitive to the status of their friendships, more exposed to a wide variety of peer stressors and receiving higher levels of emotional provisions in their friendships (Rose and Rudolph 2006). As such, peer problems may create a strong chronic stress situation in children leading to disturbed cortisol patterns.

Associations between children self-reported emotions and cortisol patterns

Although parental reported emotional problems were significant in boys, none of the scores for self-reported negative emotions (anger, sadness and anxiety) were related to cortisol. Nevertheless, self-reported happiness (positive emotion) was negatively associated with

morning and evening cortisol in boys. This suggests that also positive emotions can have an influence on cortisol concentrations. Indeed, a longitudinal study showed that positive emotions could have a protective effect on well-being (Harker and Keltner 2001). As far as we know, associations between positive affect and cortisol have not been reported in children, except for an indirect study demonstrating higher mean morning cortisol in 5-year olds that completed a story, of which only the beginning was fixed, with positive emotions, but only in girls (Hatzinger, Brand et al. 2007). In adults, Lai et al. found a negative association of evening cortisol with positive emotions and positive affect was more potent to influence cortisol secretion than its negative counterpart (Lai, Evans et al. 2005). Sex differences have been shown as positive affect was negatively associated with morning cortisol in women and with afternoon cortisol in men (Polk, Cohen et al. 2005) and positive psychosocial resources could attenuate the cortisol response to an acute stressor only in men (Kirschbaum, Klauer et al. 1995). In our study, we found only small negative correlations for self-reported happiness in boys, and no significant correlations in girls (data not shown). Since positive and negative affect are quite independent (Ryff and Singer 1998), we cannot directly infer from the significant happiness finding that elevated cortisol can be interpreted as a sign of unhappiness. Consequently, further research should elucidate these sex differences and their presence in childhood.

Associations with the CAR

Psychometric data were not associated with the CAR. A recent meta-analysis found a global positive relation of the CAR with life stress, but not with negative emotions and only a limited with positive emotions (Chida and Steptoe 2009). Nevertheless, the CAR phenomenon in our study population was difficult to examine as there was a high variability in the CAR with only half of the children having a morning increase.

Overall patterns in stress-cortisol associations

Overall, the stress-cortisol relations found in this study were more in line with the stress hypercortisolism hypothesis. Nevertheless, lower cortisol values were found in girls with peer problems. This might indicate (1) that the subjective stress assessment in this study mostly focused on recent stressors, or (2) that hypocortisolism in children is less frequent as it is mostly a consequence of chronic stress and thus time is needed to be induced. The latter might explain why there are more published hypocortisolism patterns in adolescents than in children (Bevans, Cerbone et al. 2008). Overall, both hypo- and hypercortisolism have been

demonstrated as deviations from the normal functioning and much of the variability is attributable to the stressor and person characteristics (Miller, Chen et al. 2007). As a result, inconsistency in literature could be caused by the population characteristics (sex, age and clinical sequelae), the psychometric data (time perspective, psychological concept and reporter) and the cortisol methodology (high/low cortisol may have different implications at different times of the day; used statistics).

Strengths and limitations

Important strengths of this study were the standardized procedures, the use of different questionnaires including positive affect, the use of different reporters (child and parent) and the sophisticated statistics stratified by sex. Some limitations should also be mentioned. First of all, we only collected saliva samples in the morning and evening, leaving noon and afternoon un-sampled. Nevertheless, morning and evening cortisol samples should be sufficient to study the diurnal slope (Kraemer, Giese-Davis et al. 2006). Secondly, more than two days of sampling could increase the reliability of our results, especially for the CAR (Hellhammer, Fries et al. 2007). Thirdly, we only used a subjective measure of time compliance in the salivary cortisol sampling since objective measurement of compliance was not feasible in this large population. We stressed the importance of timing and the exclusion of self-reported non-compliers can already improve the accuracy (DeSantis, Adam et al. 2010). Nevertheless, it is probable that non-compliant people are the most likely to report their timing incorrectly, which could lead to missing a part of the morning increase and as such result in the observed low CAR (Kudielka, Broderick et al. 2003). Other sampling related factors could also have an influence on the cortisol pattern, although we tried to restrict them by using an elaborated manual (Adam and Kumari 2009) and excluding the non-compliant samples (Michels, Sioen et al. 2012). Furthermore, we need to consider that the used psychometric questionnaires might not describe the whole psychological experience of these children and that over- or underestimation is possible. Nevertheless, we tried to standardize the interviewing conditions in the absence of parents and schoolteachers. Moreover, this study was cross-sectional and questionnaires covered only a short time period (last year's events, 6 month's problems, recent emotions). Analysing rather trauma of years ago might be more appropriate to unravel the hypocortisolism hypothesis (Bevans, Cerbone et al. 2008).

Conclusion

The relation between psychometric data and cortisol levels predominantly supported a possible cortisol stimulating effect of stressors in children, although lower cortisol values were detected for peer problems in girls. The strongest associations with salivary cortisol were found for happiness and peer problems. Apart from these, we have shown a relationship for stressors and emotional symptoms with the diurnal slope, emphasizing the need for slope examination. Furthermore, different associations were observed for boys and girls, suggesting that the psychometric influences on the stress-system are already sex-specific in preadolescent children. Especially, we discussed new findings on children's sex-differences in associations with peer problems and happiness. Consequently, the prevention of stress-induced pathology should focus on sex differences and also on the aspect of positive affect apart from the negative affect.

4.

Children's heart rate variability as stress indicator: association with reported stress and cortisol.

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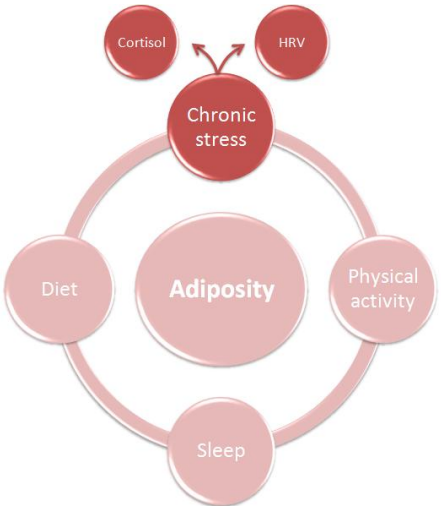


Don't let stress

break your heart.

ABSTRACT

Stress is a complex phenomenon coordinated by two main neural systems: the hypothalamic-pituitary-adrenal system with cortisol as classical stress biomarker and the autonomic nervous system. For the last system, heart rate variability (HRV) is recently suggested as stress marker. To test low HRV as stress indicator in young children, associations with self-reported chronic stress aspects and salivary cortisol were performed. Therefore, 5-minute HRV data was collected, together with salivary cortisol (4 samples/day, 2 days) (N=293) and stress related questionnaires (negative events, emotions and problems) (N=334) in children (5-10y) of the Belgian ChiBS study. Peer problems, anger, anxiety and sadness were associated with lower root mean square of successive differences (RMSSD) and high frequency power (i.e. lower parasympathetic activity). Anxiety and anger were also related to a higher low frequency to high frequency ratio. Using multilevel modelling, higher cortisol levels, a larger cortisol awakening response and steeper diurnal decline were also associated with these HRV patterns of lower parasympathetic activity. In conclusion, low HRV (lower parasympathetic activity) might serve as stress indicator in children.

Introduction

Chronic stress is linked to psychological and physiological health complaints through behavioural responses and changes in the neuroendocrine system (Cohen, Janicki-Deverts et al. 2007). Consequently, an adequate measurement of chronic stress is necessary. Stress is an adaptive, dynamic state that is composed of several aspects. The initiating stimulus is the 'stressor'. This is the environmental demand, challenge or event. When being confronted with these stressors, people evaluate whether this is a potential threat. This is the stress appraisal or perceiving phase. When homeostasis is threatened i.e. when there is a discrepancy between what is expected or the 'normal' situation and what is happening in reality, a physiological and psychological coping response will be initiated that induces arousal. Only if the person is unable to handle the persistent situation (chronic and uncontrollable stress), the sustained, chronic arousal can trigger long-term physiological, emotional, and behavioural disturbances (Ursin and Eriksen 2004, Koolhaas, Bartolomucci et al. 2011).

Next to subjective reports (by questionnaires), also biomarkers can be used. Two major neuroendocrine systems have been shown to adapt the organism to stress situations: the hypothalamus-pituitary-adrenal axis and the autonomic nervous system (Charmandari, Tsigos et al. 2005). The first system starts in the hypothalamus by secretion of the corticotrophin-releasing hormone that stimulates the pituitary which eventually stimulates the adrenal cortex to secrete cortisol as hormonal end product. The autonomic nervous system starts in the spinal cord/brainstem, results in secretion of adrenaline/noradrenaline as main hormonal end products and can be divided in the sympathetic system that prepares the body for a fight or flight and the parasympathetic system that brings to body back from an emergency status to a resting status. In times of stress, this autonomic system will give priority to cardiovascular tone and high blood pressure, respiration and release of energy substrates, while it will temporarily suppress digestion, growth, reproduction and immunity. These two major pathways can be reflected by the biomarker cortisol and heart rate variability (HRV), respectively.

Cortisol is the most commonly used biomarker for stress. Salivary cortisol sampling is stress-free and allows multiple sampling throughout the day in a natural environment. The secretion has a circadian rhythm with lowest levels around midnight and peak production in the early morning. Apart from this circadian rhythm, a cortisol awakening response (CAR) is elicited by a quick cortisol increase within 30 minutes after wake up (Fries, Dettenborn et al. 2009). In general, the CAR reflects the anticipation of the upcoming day by activation of memory

representation and by orientation in time and space. Next to the single cortisol values, these cortisol patterns (CAR and the diurnal slope) may serve as an index of adrenocortical activity on longer term. The reported associations between stress-exposure and cortisol levels are quite complex. A hyper-/hypo-cortisolism hypothesis suggested that recent exposure to a stressor may initially elevate cortisol levels (hypercortisolism with high morning cortisol and steep diurnal slope), while the axis may develop a counter-regulatory response of cortisol lowering after extended stress exposure (Heim, Ehlert et al. 2000). In a previous analysis, evidence for hypercortisolism was found in our young population since negative events and emotional problems were related to a steeper diurnal slope and low happiness with a higher overall, morning and evening cortisol, although peer problems were associated with lower overall cortisol levels in girls (Michels, Sioen et al. 2012).

Another promising and increasingly used stress marker is HRV. High HRV is defined as high variability of the distance between consecutive R peaks of the heart beat signal. This vital variability reflects the heart's ability to respond to physiological and environmental stimuli. Because of the specific autonomic nervous innervations of the heart, HRV is recognized as a quantitative marker of this autonomic nervous system: it is influenced by sympathetic activity (SA) and vagal parasympathetic activity (PA) (1996). These SA and PA innervations on the sinus node of the heart control the firing of electrical impulses that stimulate heart contraction. Importantly, the PA regularly sends inhibitory signals e.g. at expiration, with a temporary reduction of the heart rate as a result. When this PA innervations are pathologically attenuated, the sinus node will fire at its intrinsic rate, resulting in a lower variability of the heart rate i.e. low HRV. As stress influences this autonomic system, HRV can be used as an index of chronic stress, e.g. work related stress (Chandola, Heraclides et al. 2010). Low PA has previously been linked with poor emotion regulation, high stress, decreased stress reactivity and increased stress vulnerability, also in children (Porges, Doussard-Roosevelt et al. 1994, Porges 1995). Moreover, a reduction of HRV (i.e. reduced PA due to changes in the innervations with or without increased SA) is a pathway linked to higher morbidity and mortality (Thayer, Yamamoto et al. 2010) and consequently, HRV may be a potential pathway linking stress to ill health (Thayer and Brosschot 2005).

The hypothesis of the present study is that 5-minute HRV parameters (especially low PA) could serve as chronic stress indicator. Since developmental changes have been shown in HRV with age-related wave-like increases in PA (Galeev, Igisheva et al. 2002), it is

interesting to test this hypothesis also in children. After all, research on HRV as stress marker is very scarce in this population, although the prevalence of stress/mental health problems is already high in childhood (Kieling, Baker-Henningham et al. 2011) and HRV has been shown to be a risk factor for disease also in children (Zhou, Xie et al. 2012). Children's HRV will be tested as chronic stress indicator. Low HRV (=low PA) is hypothesised to be associated with (1) questionnaires on stress-related aspects and (2) hypersecretion patterns of the biomarker salivary cortisol. To cover several dimensions of stress, different questionnaires will be used. Furthermore, measuring cortisol at several time points will enable the consideration of alternative cortisol parameters across the day (overall cortisol levels, the CAR and the diurnal decline). As studies on the basal cortisol-HRV relation are almost non-existing, this paper will broaden the knowledge on the equilibrium between these two homeostasis systems.

Methods

Participants and general procedures

Participating children were recruited from the Belgian baseline ChiBS study. Detailed aims, methods and population characteristics can be found in chapter 2 "Methodology".

In total, 523 children participated in the ChiBS survey. To enable exclusion of unhealthy subjects, parents had to fill in a medical questionnaire. Concerning HRV related diseases, one child with a cardiovascular disease was excluded and no cases with diabetes were reported. Concerning cortisol, no Cushing or Addison patients were found in our population. No clinical psychopathologies were reported. Because of high quality control for HRV and cortisol data (see respective sections) and the different modules being optional, 432 children had complete HRV data, 310 had complete cortisol data and 484 had stress questionnaire data. Consequently, the analyses for HRV versus reported stress (questionnaire data) were performed in 334 children and for HRV versus salivary cortisol in 293 children. No difference in sex, parental education and physical activity was observed between children included and not included in these two sets of analyses, but those included were somewhat older.

Heart rate variability

Five-minute HRV was measured with the electrode belt Polar Wearlink in supine position. Time domain and frequency domain parameters (with the parametric AR method) were calculated.

Questionnaires on children's stress

A broad definition of stress was used by measuring the different aspects of the stress process: negative events and also outcome aspects i.e. negative emotions and behaviour. The stress appraisal phase ("are you stressed?") was not measured since this subjective representation is difficult to be answered in children. Children were individually interviewed by a trained researcher to obtain information about their life events and emotions. Furthermore, parents were asked to report on their child's behavioural and emotional problems over the past 6 months.

Life events (child-reported)

The Coddington Life Events Scale for children (CLES-C) assesses the frequency and timing of 36 stressful life events relevant for this age group during the last year (see chapter 2 "Methodology") (Coddington 1972, Coddington 1999). For this paper, the negative life events score was calculated for the last 3, 6, 9 and 12 months. Since results were similar for the different time periods, the negative event score of the last year was used for this report.

Emotions (child- reported)

Children were asked to report on their recent feelings of anger, anxiety, sadness and happiness as described in chapter 2 "Methodology".

Problems (parent- reported)

Parents were asked to complete 4 subscales of the 'Strengths and Difficulties Questionnaire' (Goodman 1997) on problems of their child over the past 6 months: emotional problems, conduct problems, peer problems and prosocial behaviour. Higher scores on the prosocial behaviour subscale reflect strengths, whereas higher scores on the other three subscales reflect problems.

Salivary cortisol

Saliva was collected at home via Salivette synthetic swabs (Sarstedt, Germany) immediately after wake up (T0), 30 minutes after wake up (T30), 60 minutes after wake up (T60) and in the evening between 7 and 9 PM (Tev). More details on sampling and analyses can be found in chapter 2 "Methodology".

Possible confounders

Parental reported physical activity, parental education, children's sex and age were used as possible confounders. Details can be found in chapter 2 "Methodology".

Statistical analyses

Most statistical analyses were performed using SPSS/PASW version 19 (IBM Corp, NY, USA), while the cortisol-HRV regression was performed in the multilevel HLM/2L program (version 7.0) using an approach similar to the one published by Adam (2006). The significance level was always set to $p<0.05$. Due to a skewed distribution, HF power, LF power, LF/HF and cortisol concentrations were log-transformed. Chi-square statistic and Mann-Whitney U statistic were used to test the selection bias for categorical (sex and parental education) and continuous variables (age and physical activity) respectively by comparing the included and excluded population. Mann-Whitney U and spearman correlation were used in detecting sex and age differences (age used as a continuous parameter) in physical activity, HRV, questionnaire and cortisol parameters.

Linear regression was used to analyse the relation between the children's HRV (dependent variable) and stress questionnaire data: (1) the emotions happy, angry, anxious and sad, (2) the negative event score during the last 12 months and (3) emotional problems, conduct problems, peer problems and prosocial behaviour. The analyses were adjusted for age, sex and physical activity as these were important HRV determinants in this population sample (Michels, Clays et al. 2012). Analyses were stratified by sex when a significant interaction with sex was present.

Hierarchical linear modelling was used to analyse the relation between the children's cortisol pattern and their HRV, adjusted for potential confounders (age, sex, physical activity and wake up time). It is a variant of multiple linear regression useful for data with a nested design, which is the case in this study as repeated cortisol measurements were obtained for each participating child. Therefore, a two-level model on the dependent variable 'cortisol' was used with the intra-individual parameters modelled at level 1 (cortisol pattern created by time of day) and the inter-individual parameters (HRV data and the personal characteristics as possible confounders) at level 2. At level 1, the child's cortisol values were predicted by the time of day, to estimate the shape of each child's cortisol curve during the day. Time of day values were expressed as 'number of hours since wake up' for each participant each day and centred to midday as six hours post-awakening. To characterise the CAR, a design variable

was created assigning the value 1 to the sample taken 30 minutes after wake up, and the value 0 to all other samples. By representing the CAR as a separate variable in the model, the CAR became a separate coefficient that could be predicted independently of the other parameters, such as the diurnal slope. A dummy variable for day of measurement (first or second day) was included to account for possible systematic cortisol differences across days. The reported day-specific wake up time, considered as a cortisol-confounder, was also included as a level 1 parameter because of its day-dependence. HRV parameters were entered as a level 2 predictor of each of the relevant level 1 predictors (intercept, slope and CAR) and these models were adjusted for potential confounders (age, sex, physical activity and wake up time). Because of multicollinearity, the HRV parameters were not entered simultaneously.

Results

Information was available on both HRV and salivary cortisol for 293 children (50.5% boys) and information on both HRV and stress questionnaires for 334 children (51.2% boys). The children were almost evenly distributed over the age range 5 to 10. Tertiary parental education was seen in 71.4%. Descriptive statistics on the used variables are shown in Table 15. Sex differences were found in HRV, stress questionnaires and physical activity parameters with boys having higher HRV, more physical activity and more conduct problems, while girls having a higher mean heart rate and reporting more anxiety and sadness. HRV parameters representing PA, cortisol values, physical activity and self-reported anger increased by age, while the other HRV parameters and self-reported happiness decreased.

HRV versus stress related questionnaire data.

In Table 16, HRV parameters were tested for associations with questionnaire data on stress aspects. The total negative event score, happiness, prosocial behaviour and emotional and conduct problems were not associated with HRV (data not shown). Sex interaction effects were found for the effect of anxiety (on RMSSD p=0.010; on pNN50 p=0.043, on HF p=0.008; on HFnu p=0.022), sadness (on pNN50 p=0.041; on HF p=0.029) and peer problems (for RMSSD p=0.007; for HF p=0.025): associations for anxiety and sadness were observed only in girls and for peer problems only in boys. Anger, anxiety, sadness and peer problems were all associated with lower PA (lower RMSSD and HF), while anxiety was also related to higher LF/HF, perhaps due to a parallel increased LFnu.

Table 15: Descriptive statistics and sex and age differences for heart rate variability, physical activity, stress questionnaire data and salivary cortisol levels

	Median	Interquartile range	Sex	Age
Heart rate variability (N=432)				correlation coefficient
Mean heart rate	82	[75 - 98]	B<G	-0.415*
Time domain parameters				
RMSSD (ms)	71	[53 – 100]	B>G	0.141*
pNN50 (%)	41	[27 – 55]	B>G	0.207*
Autoregression				
LF power (ms^2)	443	[239 – 800]	B>G	0.031
HF power (ms^2)	428	[224 – 787]	B>G	0.161*
LFnu (normalized units)	47	[38 – 56]		-0.161*
HFnu (normalized units)	46	[34 – 56]		0.211*
LF/HF ratio	1.02	[0.71 – 1.58]		-0.209*
Physical activity (N=432) (self reported hours/week)	14	[10 – 20]	B>G	0.115*
Stress questionnaire data (N=334)				
Emotions				
Happiness (Likert scale 0-10)	8	[6 – 10]		-0.221*
Anger (Likert scale 0-10)	3	[1 – 5]		0.126*
Anxiety (Likert scale 0-10)	1	[0 – 3]	B<G	-0.039
Sadness (Likert scale 0-10)	2	[0 – 4]	B<G	0.008
Negative event score last 12 months	39	[7 – 72]		0.094
Strengths and Difficulties Questionnaire				
Emotional problems (0-10)	2	[1 – 4]		-0.042
Conduct problems (0-6)	1	[0 – 2]	B>G	-0.052
Peer problems (0-10)	1	[0 – 2]		-0.017
Prosocial behaviour (0-8)	7	[6 – 8]	B<G	0.076
Salivary cortisol (N=293)				
Immediately after wake up (nmol/l)	12.11	[9.74 – 14.47]		0.129*
30 minutes after wake up (nmol/l)	12.77	[9.90 – 15.66]		0.124*
60 minutes after wake up (nmol/l)	7.93	[5.96 – 11.00]		0.116*
Evening (nmol/l)	1.63	[1.17 – 2.44]		0.181*

*HF= high frequency spectral power; LF= low frequency spectral power; LF/HF= ratio of low frequency power to high frequency power; pNN50= percentage of consecutive normal RRI differing more than 50 ms; RMSSD= root mean square of successive differences; * p<0.05; B= boys; G= Girls*

Table 16: Linear regression predicting heart rate variability parameters by stress questionnaire data adjusted for age, physical activity and sex or stratified by sex (N=334)

		Time-domain HRV parameters		Autoregression HRV parameters				
		RMSSD [ms]	pNN50 [%]	LF [ms²]	HF [ms²]	LFnu	HFnu	LF/HF
Emotions								
Anger (both sexes)[a]	bèta	**-0.111**	0.072	-0.052	**-0.147**	0.098	**-0.121**	**0.114**
	p	**0.037**	0.174	0.316	**0.006**	0.070	**0.022**	**0.036**
Anxiety (boys)	bèta	0.080	0.063	0.097	0.043	0.095	-0.043	0.073
	p	0.293	0.403	0.206	0.573	0.213	0.572	0.337
Anxiety (girls)	bèta	**-0.219**	**-0.160**	-0.120	**-0.250**	**0.190**	**-0.223**	**0.208**
	p	**0.006**	**0.040**	0.131	**0.002**	**0.019**	**0.008**	**0.009**
Sadness (boys)	bèta	-0.024	-0.018	0.011	-0.001	0.054	0.007	0.017
	p	0.754	0.809	0.891	0.991	0.480	0.927	0.821
Sadness (girls)	bèta	**-0.200**	**-0.220**	-0.127	**-0.195**	0.118	-0.112	0.118
	p	**0.012**	**0.006**	0.111	**0.014**	0.138	0.151	0.132
Strengths and Difficulties Questionnaire								
Peer problems (boys)	bèta	**-0.164**	-0.129	**-0.166**	**-0.187**	0.046	-0.064	0.060
	p	**0.029**	0.085	**0.034**	**0.017**	0.541	0.383	0.412
Peer problems (girls)	bèta	-0.008	0.031	-0.019	0.058	-0.096	0.112	-0.105
	p	0.917	0.704	0.809	0.472	0.232	0.156	0.182

HF= high frequency spectral power; LF= low frequency spectral power; LF/HF= ratio of low frequency power to high frequency power; nu= normalized units; pNN50= percentage of consecutive normal RRI differing more than 50 ms; RMSSD= root mean square of successive differences; [a]= not stratified by sex since the sex interaction factor was not significant.

HRV versus cortisol

Figure 22 illustrates the significant associations between salivary cortisol and HRV. Both overall cortisol levels, the diurnal cortisol slope and the CAR were associated with HRV. Higher overall cortisol levels and a steeper diurnal decline were negatively associated with the PA markers RMSSD, pNN50 and HF. A larger CAR was associated with a higher LFnu and LF/HF ratio.

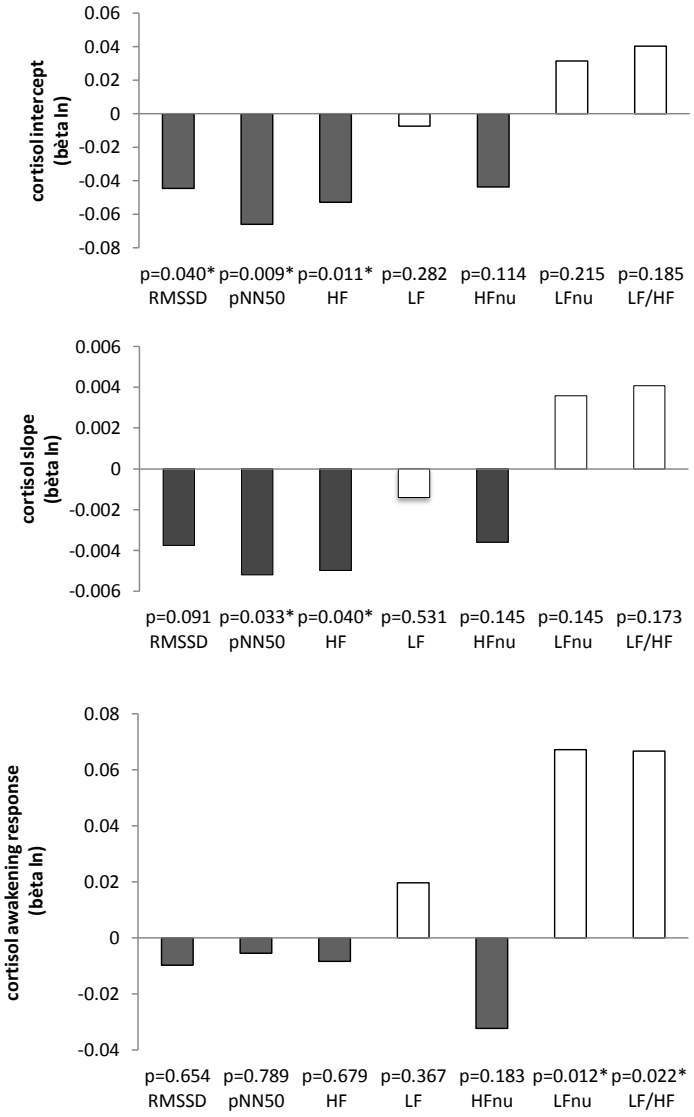


Figure 22: Associations between children's cortisol and heart rate variability adjusted for age, sex, physical activity and wake up time (N=293).

Standardized coefficients (in natural logarithm) of different cortisol patterns are shown: cortisol intercept (panel A), cortisol diurnal slope (panel B) and cortisol awakening response (panel C). Filled bars are the heart rate variability parameters representing the parasympathetic activity, empty bars are those representing the sympathetic activity or a combination of both parasympathetic and sympathetic activity.

HF= high frequency spectral power; LF= low frequency spectral power; LF/HF= ratio of low frequency power to high frequency power; nu= normalized units; pNN50= percentage of consecutive normal RRI differing more than 50 ms; RMSSD= root mean square of successive differences.

Discussion

Our results in 5-10 year old children are in line with the suggestion of low HRV (=low PA) as stress indicator by its reflection of chronic stress-induced changes in the autonomic nervous system (1996, Thayer and Brosschot 2005).

First of all, low PA and possibly also high SA was related to higher reports of certain stress aspects in our child population: peer problems, anger, anxiety and sadness were associated with lower PA. Anxiety was also associated with higher LFnu and LF/HF that may reflect more sympathetic over parasympathetic dominance. Since a reduction of HRV (i.e. reduced PA with or without increased SA) is a pathway of increased morbidity and mortality (Thayer, Yamamoto et al. 2010), HRV may be a potential pathway linking stress to ill health (Thayer and Brosschot 2005).

Furthermore, HRV showed moderate associations with the other stress marker cortisol, although both represent two different neural stress systems: the autonomic nervous system and the hypothalamus-pituitary-adrenal axis, respectively. Higher salivary cortisol levels and diurnal slopes were associated with lower PA. Larger CAR was associated with higher LFnu and LF/HF: a higher sympathetic over parasympathetic dominance might be caused by an increased SA since PA parameters were not significantly decreased.

When comparing the relations of the different HRV parameters, HF and RMSSD were most significant in the relation with stress questionnaires and HF and pNN50 were most significant in relation with cortisol. Consequently, this confirms our hypothesis that low PA can indicate stress. HF could be the preferred stress indicator. Indeed, Porges et al. have previously stated that especially low vagal tone (PA) is linked with poor emotion regulation, high stress, decreased stress reactivity and increased stress vulnerability, even in early life (Porges, Doussard-Roosevelt et al. 1994, Porges 1995).

HRV versus stress questionnaire data

In a previous publication, significant associations were found between salivary cortisol and our stress questionnaire data namely the negative event score, emotional problems, peer problems and happiness (Michels, Sioen et al. 2012). In the current publication, we confirm that low HRV (low PA) can be predicted by peer problems, although the HRV associations were most prominent for the negative emotions. More specifically, evidence was highest for the negative emotion ‘anxiety’: anxiety was related with lower PA parameters (RMSSD,

pNN50, HF and HFnu) and a higher LFnu and LF/HF ratio. Consequently, anxiety was related with a lower PA and a lower sympathetic over parasympathetic dominance (possibly due to a concurrently higher SA). Indeed, a vagal circuit (PA) in the physiological regulation of emotions has previously been outlined (Porges, Doussard-Roosevelt et al. 1994). Literature is especially abundant on the HRV relationship with anxiety, e.g. anxiety in adults and children was related to both low PA and high SA in a recent review (Friedman 2007). Peer problems are not a direct measurement of stress itself. Nevertheless, they can elicit chronic stress by the long-lasting exposure to bullying and by threatening the establishment of close and enduring interpersonal relationships (Baumeister and Leary 1995). Since the arousal will sustain until the reason for the arousal is eliminated, these difficult situations will not lead to phasic arousal but to sustained, chronic arousal.

HRV could not be predicted by the negative event score. Since the event questionnaire only measured the occurrence of events and not the subjective stress perception or coping, it is not known whether the reported events were perceived as stressful and how the children dealt with it (Miller, Chen et al. 2007). Consequently, this could have reduced the ability to observe stronger associations. Although HRV could theoretically be influenced by behavioural factors like sociality and conduct disorders (Beauchaine 2001), HRV could not be predicted by prosocial behaviour, emotional problems and conduct problems in our study.

The observed stress-HRV relations showed sex differences for certain questionnaire data :anxiety and sadness were only related to HRV parameters in girls, while peer problems were only related to HRV parameters in boys. Underlying mechanisms could be the sex differences that have been demonstrated in the used HRV parameters (Michels, Clays et al. 2012), in psychological functioning and development of children (Crick and Zahn-Waxler 2003) and in handling stressful situations (Hampel and Petermann 2005). Sex differences in HRV-anxiety have also been reported with significances only in women and girls (Grossman, Wilhelm et al. 2001, Greaves-Lord, Tulen et al. 2010). This phenomenon occurs also in other stress relations as our happiness and peer problem data was in a sex-dependent manner related to cortisol in a previous analysis (Michels, Sioen et al. 2012). Consequently, sex differences should always be considered in stress measurements.

HRV versus cortisol

Since both HRV and cortisol have been shown to reflect subjective stress reports, the second hypothesis was that HRV and cortisol could show good agreement in their status. Low HRV (i.e. low PA) was indeed related to specific cortisol patterns that have previously been associated with stress questionnaires in our population, namely a steeper diurnal decline and sometimes higher cortisol values (Michels, Sioen et al. 2012). Low HRV (a higher sympathetic over parasympathetic dominance) was in our study also related to a higher CAR which was positively associated with life stress in a recent meta-analysis (Chida and Steptoe 2009). Up to now, only few studies examined the HRV-cortisol relation and those handled particularly the CAR. Higher CAR values have been associated with low HF and LF but no changes in LF/HF cross-sectionally (Stalder, Evans et al. 2011) and with low HF longitudinally (Eller 2007). Nevertheless, also non-significant findings have been published (Gunnar, Porter et al. 1995, Johnson, Hansen et al. 2002). Studies have also been done on parallelism in stress reactions of these two biomarkers. A natural occurring stressor of examination could both increase cortisol and decrease HRV in students (Sgoifo, Braglia et al. 2003), as was the case for a laboratory stressor in children (Doussard-Roosevelt, Montgomery et al. 2003). Furthermore, the baseline HF status might determine the cortisol response to stress, although there are inconsistent findings (Gunnar, Porter et al. 1995, Johnson, Hansen et al. 2002, Smeets 2010).

Despite the shown associations between HRV and cortisol, their reaction on stress may still differ. For instance, a dissociation has been reported in the reactivity of both neural systems to repeated stressors with a lower cortisol stress response after several repeated exposures (=habituation), while the HRV reaction remained high (Schommer, Hellhammer et al. 2003). Besides, the differential associations of HRV and cortisol with stress questionnaire data (as we have shown here) may indicate activation differences in the systems underlying both biomarkers. As a result, a combination of cortisol and HRV measurements could be considered when examining population differences in stress status. When already implementing salivary cortisol measurements, the HRV measurements do not add high additional burden to the examiner and the participant as the measurement is very short and is well accepted by the children.

Strengths and limitations

This paper contributes to the scarce literature linking HRV and cortisol. Important strengths of this study are (1) the standardized and quality controlled HRV measurements with several calculated parameters, (2) the comparison of HRV with both questionnaire data and cortisol, (3) the use of sophisticated analyses with correction and stratification, (4) the use of different questionnaires to cover the different aspects of stress and (5) the repeated cortisol measures enabling the consideration of several alternative parameters across the day.

Concerning the questionnaires, we need to consider that over- or underreporting of emotions or problems is possible since these are subjective data and that a broad definition of stress has been used. Nevertheless, we tried to standardize the interviewing conditions in the absence of parents and schoolteachers. Concerning salivary cortisol, we only used a subjective measure of time compliance in the cortisol sampling since objective compliance measurements were not feasible in this large population. Nevertheless, accuracy was improved by stressing the importance of timing and excluding self-reported non-compliers (DeSantis, Adam et al. 2010). Concerning HRV, measures were only performed in supine position. Since supine position could diminish SA, future research should also test basal HRV measures in sitting position as marker of chronic stress (Vybiral, Bryg et al. 1989). Although visual respiratory observation was done during the registration, breathing rate was not measured with an extra channel. Breathing rate was shown to be negatively related with LF and HF power, but had no influence on time-domain parameters (Brown, Beightol et al. 1993, Penttila, Helminen et al. 2001). Nonlinearities are more likely to happen with a low breathing rate (e.g. at 10/minute breathing rate), a long RRI and in the presence of important respiratory sinus arrhythmia (Porta, Baselli et al. 2000). Nevertheless, the influence of nonlinearities will be minimal in our population as children have a general high breathing rate (18-21/minute) and short RR-interval; and respiratory sinus arrhythmia induced by the Valsalva manoeuvre was more likely abolished. Furthermore, the measurement modules were optional resulting in smaller samples when combining HRV with cortisol or questionnaire data, although our high quality control was partially responsible for this. Finally, the cross-sectional nature of our results limits the directional statements on the relationships. Although our hypothesis was that chronic stress may result in a simultaneous effect on HRV and cortisol status, interconnections between the two pathways exists since corticotrophin-releasing factor and noradrenalin can stimulate each other directly or indirectly e.g. vagal PA innervations of the amygdala influence cortisol (Thayer and Brosschot 2005, Thayer and Sternberg 2006).

Conclusion

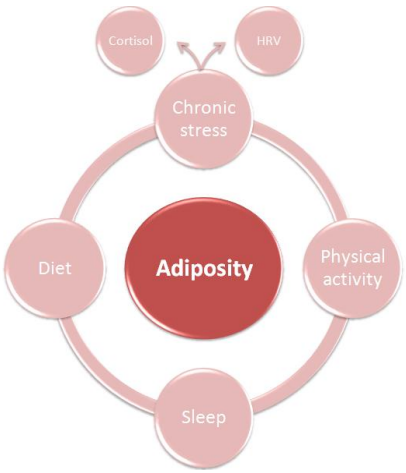
We have shown that children's HRV (especially low parasympathetic activity) might serve as a stress indicator since it reflects some aspects of their stress status as represented by questionnaire data on negative emotions and peer problems. Moreover, HRV showed moderate associations with cortisol, the marker of the other main neural stress system i.e. the hypothalamus-pituitary-adrenal axis. Nevertheless, measuring both pathways is recommended as they might be stimulated differently depending on the stressor or stress outcome. Moreover, further research is necessary to proof HRV as a stress indicator because of inherent problems in assessing complex concepts (adverse life events and emotions) among young children.

5.

Caucasian children's fat mass: routine anthropometry versus air-displacement plethysmography.

Michels N, Huybrechts I, Bammann K, Lissner L, Moreno L, Peeters M, Sioen I, Vanaelst B, Vyncke K, De Henauw S.

British Journal of Nutrition 2013; 109 (8): 1528-37.



I am not overweight,

I am just some inches too short.

ABSTRACT

This paper will use fat mass percentage obtained via BOD POD® air-displacement plethysmography (FM_{ADP}%) to examine the relative validity of 1) anthropometric measurements/indices and 2) of fat mass percentage assessed with equations (FM_{eq}%) based on skinfold thickness and bioelectrical impedance. In 480 Belgian children (5 to 11 years old) weight, height, skinfold thickness (triceps and subscapular), body circumferences (mid-upper arm, waist and hip), foot-to-foot bioelectrical impedance (Tanita®) and FM_{ADP}% were measured. Anthropometric measurements and calculated indices were compared with FM_{ADP}%. Next, published equations were used to calculate FM_{eq}% using impedance (equations of Tanita®, Tyrrell, Shaefer and Deurenberg) or skinfold thickness (equations of Slaughter, Goran, Dezenberg and Deurenberg). Both indices and equations performed better in girls than in boys. For both sexes, the sum of skinfold thicknesses resulted in the highest correlation with FM_{ADP}%, followed by triceps skinfold, arm fat area and subscapular skinfold. In general, comparing FM_{eq}% with FM_{ADP}% indicated mostly an age and sex effect and an increasing underestimation but less dispersion with increasing FM%. The Tanita® impedance equation and the Deurenberg skinfold equation performed the best, although none of the used equations were interchangeable with FM_{ADP}%. In conclusion, the sum of triceps and subscapular skinfold thickness is recommended as marker of FM% in the absence of specialized technologies. Nevertheless, the higher workload, cost and survey management of an immobile device like the BOD POD® remains justified.

Introduction

Air-displacement plethysmography (ADP), integrated in the commercially available system BOD POD® is a validated technique to assess body composition (Fields, Goran et al. 2002). It has the advantage over the four-compartment model of including a quick, comfortable, automated, non-invasive and safe measurement process, making it feasible for children. As the best performing two-compartment model, ADP is more reliable for body composition than routine anthropometric measurements (Fields and Goran 2000). Nevertheless, the conversion from body density obtained by ADP measurement to fat mass percentage (FM_{ADP}%) needs consideration in children. As the chemical maturation of lean tissue changes with age and proceeds differently in males and females, age- and sex-adjusted factors need to be considered for children as was done by Lohman and more up-to-date by Wells (Lohman 1989, Wells, Williams et al. 2010).

Although ADP is considered as a more feasible method for large scale surveys in comparison with the four-compartment model, the immobile aspect of ADP could also be a limitation for large scale surveys. Due to these logistic and budgetary constraints, examinations in large scale epidemiological studies including the assessment of body composition are often restricted to only routine measurements (weight, height, circumferences, skinfold thickness and bioelectrical impedance (BIA)). However, the accuracy of routine anthropometric measurements in children is still doubted, although inter- and intra-observer error can be controlled when thorough training is carried out (Stomfai, Ahrens et al. 2011). Furthermore, a variety of techniques are used (e.g. whole body vs foot-to-foot BIA) and equations to convert the measurement in FM% are population-specific. Especially in children, the assessment of body composition remains a challenging task (Wells and Fewtrell 2006, Sweeting 2007).

In this paper, the validity of anthropometric measurements/indices and of FM% equations (FM_{eq}%) based on anthropometric measurements (skinfolds and BIA) was investigated with FM_{ADP}%, using as reference method the BOD POD® device with the up-to-date Wells adjusting factors for children. As such, research groups without specialized technologies such as ADP can make a well-founded choice on the routine anthropometric measurements, indices and equations to be used in obtaining a good indication of children's body fat. Furthermore, it is interesting to examine whether the bigger workload, cost and the more complicated survey

management with an immobile device like the BOD POD® is justified in large-scale surveys (i.e. are the anthropometric measurements interchangeable with ADP). This study will help in these two decisions using a large child population (n=480) in which a large battery of anthropometric measurements was performed.

Methods and materials

Population

Participating children were part of the Belgian baseline ChiBS survey. 480 children (52.3% male, 6.7% overweight) between 5-11 years old underwent the ADP measurement. Detailed aims, methods and population characteristics can be found in chapter 2 "Methodology".

Body composition measurements

Reference method: ADP

FM_{ADP}% was measured with ADP using the up-to-date child-specific conversion factors reported by Wells as reported in chapter 2 "Methodology".

Routine anthropometric measurements

The children were measured barefooted in underwear and/or T-shirt.

Raw anthropometric data from weight, height, leg-to-leg impedance, triceps skinfold (TSF), subscapular skinfold (SSF) and body circumferences were obtained as described in chapter 2 "Methodology".

Equations found in literature were used to calculate FM_{eq}% as long as they were at least partly based upon a population sample that included children and if all needed parameters were measured in this study. For BIA, only equations using the impedance (as we have measured), and not the resistance or reactance were selected. The selected equations based on impedance or skinfold thickness and their characteristics are listed in Table 17. Apart from the built-in Tanita® equation (Susan, McCarthy et al. 2004), the equations of Schaefer (Schaefer, Georgi et al. 1994), Deurenberg 1 and 2 (Deurenberg, Pieters et al. 1990, Deurenberg, van der Kooy et al. 1991) and Tyrrell (Tyrrell, Richards et al. 2001) were selected for the impedance measurements and the equations of Slaughter (Slaughter, Lohman et al. 1988), Goran (Goran, Driscoll et al. 1996), Deurenberg 3 (Deurenberg, Kusters et al. 1990) and Dezenberg (Dezenberg, Nagy et al. 1999) for skinfold measurements.

Table 17: Fat percentage assessing equations investigated in this study

Source	Age	Ethnicity	FM% (mean+-SD)	N	Reference method	BIA Type	Variables	Times cited	Equations
Bioelectrical impedance (BIA)									
Tanita®	4-18 y	England	NM	101	DEXA	F	I, W, H, S, A	/	Not published
Tyrrell	5-11 y	Europe, Maori, pacific island	28.1+-9.8	82	DEXA	F	I, S, W, H	45	FFM= 0.31H²/I + 0.17H + 0.11W + 0.942S - 14.96 (girls=1; boys=2)
Schaefer	3-19 y	Germany	19.6+-8.1	112	K spectrometry	Wh	I, H, A	124	FFM= 0.65 (H²/I) + 0.68A + 0.15
Deurenberg 1	7-25 y	Netherlands	boys 18.7+-5.8 girls 20.4+-7.0	246	Underwater weighing	Wh	I, A, (W), H	146	Younger than 10 years: FFM= 0.640H²/I + 4.83 10-12(girls), 15(boys): FFM= 0.488H²/I + 0.221W + 0.1277H - 14.7
Deurenberg 2	7-15 y	Netherlands	24.6+-9.1	166	Underwater weighing	Wh	I, W, H, S	185	FFM= 0.406H²/I + 0.36W + 0.56S + 0.0558H - 6.5 (boys=1, girls=0)
Skinfolds									
Slaughter	8-29 y	US, white and black	boys 19+-8.1 girls 23.3+-6.6	310 (66 preadolescent)	Four-compartment		TSF, SSF, W, S, M	754	boys SFS>35 FM%=0.783SFS + 1.6 girls SFS>35 FM%= 0.546SFS + 9.7 other boys white FM%= 1.21SFS - 0.008SFS²) - 1.7 other boys black FM%= 1.21SFS - 0.008SFS²) - 3.2 other girls FM%= 1.33SFS - 0.013SFS² -2.5
Goran	4-9 y	US, almost all white	NM	98	DEXA		TSF, SSF, W	173	FM= 0.23SSF + 0.18W + 0.13TSF - 3
Dezenberg	4-11 y	Caucasian and African-American	[6.2-49.6]†	202	DEXA		TSF, W, S, E	35	FM= 0.38W + 0.30TSF + 0.87S + 0.81E - 9.42 (Caucasian=1, African-American=2, boys=1, girls=2)
Deurenberg 3	7-20 y	Netherlands	15.8+-0.54‡	378 (98 preadolescent)	Underwater weighing		TSF, SSF, S	146	boys FM%= -14.61 + 26.51logSFS girls FM%= -16.84 + 29.30logSFS

FM%= fat mass percentage (mean +- standard deviation); NM= not mentioned; F= foot-to-foot BIA; I= impedance; W= weight [kg]; H= height [cm]; S= sex; A= age; FFM= fat free mass [kg]; Wh= whole body BIA; TSF= triceps skinfold [mm]; SSF= subscapular skinfold [mm]; M= sexual maturation; SFS= skinfoldsum (= TSF+SSF) [mm]; FM= fat mass [kg]; E= ethnicity; † range; ‡ mean +- standard error

Indices. Apart from BMI (BMI=weight(kg)/height(m)²), also Rohrer's Index (RI= weight[kg]/height[m]³), arm fat area (AFA= (MUAC[cm]²/4pi) - (MUAC[cm]-(pi*TSF[cm]))²/4pi)), waist-to-hip ratio (WHR= waist[cm]/hip[cm]), waist-to-height ratio (WHtR= waist[cm]/height[cm]), the Conicity Index (ConI= waist[cm]/(0.109* $\sqrt{weight[kg]/height[cm]}$) and *impedance index* (=height²/impedance) were calculated to assess the correlation between these indices and the FM_{ADP}%.

Statistical analyses

Means and standard deviations were given for all measurements and the association with sex and age was tested. As indices were not normally distributed, Mann-Whitney U tests and Spearman's correlation coefficients were employed.

For the first objective, the correlation between anthropometric measurements/indices and FM_{ADP}% was determined using age-adjusted Spearman correlations.

The second objective was to compare FM_{eq}% using skinfold measurements or BIA (see Table 17) with FM_{ADP}%. First of all, mean difference +/- SD between FM_{ADP}% and FM_{eq}% was given; its significance was determined with paired t-test using Bonferroni adjustment. As the difference is calculated as 'FM_{ADP}% minus FM_{eq}%', a positive mean difference indicates an underestimation by FM_{eq}%. To compare these results with those of the anthropometric indices, age adjusted correlations were calculated.

Accuracy and precision were examined using regression analysis. As nine equations were tested separately for boys and girls, the results of 18 regression analyses were noted. If age was a significant predictor, the results of the multiple regression were given (both FM_{eq}% and age as predictors), otherwise of the simple regression. The FM_{eq}% was considered accurate when the regression between FM_{ADP}% and FM_{eq}% did not differ significantly from the line of identity (slope not significantly different from 1 and intercept not significantly different from 0). The precision of FM_{eq}% was assessed by the R^2 and the standard error of the estimate (SEE).

The presence of a sex-effect on the FM% difference was separately examined by multiple regression analyses with each time one of the nine FM_{eq}% and sex as predictors. To examine whether there was a real sex effect or whether this was induced due to the FM% difference between sexes, a FM_{eq}%-sex interaction term was included as an extra variable in this regression.

Bland-Altman analysis was used to examine the agreement between FM_{eq}% and FM_{ADP}%. Apart from the 95% limits of agreement (LOA), also the presence of a direction (=heteroscedacity: upwards/downwards) or dispersion (convergent/divergent) trend across the range of fatness were examined. A downwards or upwards direction trend was present if there was a significant univariate regression line between "FM_{ADP}%-FM_{eq}%" and the mean values of FM_{ADP}% and $FM_{eq\%}$. A potential dispersion trend (convergent/divergent from the zero point) was visually evaluated. As the difference was calculated as 'FM_{ADP}% minus FM_{eq}%', an upwards Bland-Altman line indicates an underestimation by FM_{eq}% in children with a higher fat mass (if the Bland-Altman regression line goes through the zero point). A convergent dispersion trend demonstrates more FM% agreement between both methods with increasing FM%, while a divergent trend shows more disagreement with increasing FM%.

Finally, single measure intraclass correlation (ICC) was calculated as interchangeability measure using a two-way mixed model with absolute agreement. Interchangeability has been suggested to be excellent when the ICC coefficient is higher than 0.75.

All statistical analyses were performed using SPSS/PASW version 19 (IBM Corp, USA). To correct for multiple testing when using the FM% equations, a Bonferroni correction was applied with a *p*-value of 0.003 (0.05/18; 9 equations*2 sexes= 18 tests) as the threshold of significance. For all other analyses, the two-sided level of significance was set at $p<0.05$.

Results

Descriptive data on the study population is shown in Table 18. Boys and girls were not significantly different in age. Girls had a higher FM_{ADP}%, arm circumference, skinfold thickness, AFA and RI, but a lower impedance index and a lower waist-to-hip ratio. For measurement results, anthropometric values increased with age, except for a negative trend in impedance and no trend in FM_{ADP}%. Based on the BMI z-scores, 6.7% of our population was overweight, with FM_{ADP}% ranging between 6.27 - 37.35% for boys and 9.31 - 45.14% for girls.

Table 19 gives the age-adjusted Spearman correlations between FM_{ADP}% and calculated indices stratified by gender. All correlations were highly significant except for WHR and impedance index in boys. All correlations were higher in girls than in boys. For both sexes, sum of both skinfold thicknesses showed highest correlation, followed by TSF, AFA and SSF. The lowest correlation coefficients were found for ConI, WHR and impedance index. The

remaining indices (RI, BMI, BMI z-score, WHtR, MUAC, 'weight minus impedance index', hip and waist) had similar intermediary correlations (coefficients between 0.447 and 0.699).

Some indices were calculated using the same measurement data. First of all, WHtR performed better than WHR and ConI and slightly better than waist circumference. Secondly, RI and BMI had similar correlational strength. Thirdly, AFA gave very similar results as the TSF, but somewhat better than MUAC. Finally, 'weight minus impedance index' was preferred to the impedance index itself. This result of 'weight minus impedance index' was not caused purely by the correlation of weight with FM%, as the latter was only 0.148 and 0.393 in boys and girls respectively (data not shown in table).

Table 18: Descriptive statistics for the study population and age and sex differences

	Male (n=251)		Female (n=229)			
	Mean	SD	Mean	SD	Sex [a]	Age [b]
Measurement results						
Age [years]	7.93	1.58	7.85	1.49	NS	N/A
Height [cm]	129.27	10.79	127.87	10.17	NS	0.848*
Weight [kg]	26.61	5.96	26.53	6.30	NS	0.767*
Mid-upper arm circumference [cm]	19.25	1.98	19.93	2.39	*	0.495*
Hip circumference [cm]	65.60	6.15	66.85	6.86	NS	0.688*
Waist circumference [cm]	56.26	5.09	56.75	6.69	NS	0.504*
Triceps skinfold [mm]	9.28	2.85	11.80	4.22	*	0.146*
Subscapular skinfold [mm]	5.75	1.80	7.41	3.78	*	0.122*
FM_{ADP}% [%]	16.40	5.37	19.43	6.77	*	-0.046
Impedance [ohm]	629.68	62.97	676.71	71.15	*	-0.240*
Indices						
Body Mass Index [kg/m2]	15.73	1.55	16.03	2.13	NS	0.235*
BMI z-score (Cole method)	-0.21	0.91	-0.09	1.15	NS	0.052
Rohrer's index [kg/m3]	12.19	1.31	12.56	1.66	*	-0.404*
Sum of both skinfolds [mm]	14.91	4.31	19.17	7.43	*	0.150*
Arm fat area [cm2]	8.29	3.26	10.89	5.09	*	0.259*
Conicity index	1.14	0.04	1.15	0.06	NS	-0.061
Waist-to-hip ratio	0.87	0.04	0.84	0.05	*	-0.374*
Waist-to-height ratio	0.44	0.03	0.44	0.04	NS	-0.364*
Impedance index (height²/impedance) [cm²/ohm]	27.10	5.76	24.71	5.35	*	0.733*
Weight minus impedance index	-0.57	2.80	1.76	2.86	*	0.462

*NS= not significant; N/A= not applicable; FMADP%= fat mass percentage with air-displacement plethysmography; [a] Mann Whitney U test; [b] Spearman correlation coefficient; *significant at p<0.05*

Table 19: Age-adjusted Spearman correlations of anthropometric measurement data and indices with BOD POD fat mass percentage, stratified by sex

	Male (n=251)		Female (n=229)	
	r	p	r	p
Measurement results				
Mid-upper arm circumference	0.501	<0.001	0.661	<0.001
Hip circumference	0.447	<0.001	0.635	<0.001
Waist circumference	0.455	<0.001	0.633	<0.001
Triceps skinfold	0.670	<0.001	0.766	<0.001
Subscapular skinfold	0.635	<0.001	0.728	<0.001
Indices				
Body mass index	0.511	<0.001	0.699	<0.001
Body mass index z-score	0.500	<0.001	0.679	<0.001
Rohrer's index	0.519	<0.001	0.651	<0.001
Sum of both skinfolds	0.710	<0.001	0.815	<0.001
Arm fat area	0.670	<0.001	0.762	<0.001
Conicity index	0.215	0.001	0.380	<0.001
Waist-to-hip ratio	0.113	0.075	0.227	0.001
Waist-to-height ratio	0.511	<0.001	0.647	<0.001
Impedance index	0.085	0.185	0.284	<0.001
Weight minus impedance index	0.494	<0.001	0.608	<0.001

r= correlation coefficient

In Table 20, FM_{eq}% of published equations were tested against FM_{ADP}%. Overall, better results were obtained in girls, although no real sex difference existed when ranking on validity.

First, we investigated the sex and age effects. Analyses were stratified by sex as several FM_{eq}% (Tanita®, Tyrrell, Shaefer, Deurenberg 2 and Deurenberg 3) showed a significant sex effect (data not shown). After correction for the FM%-sex interaction, the sex effect disappeared in the Deurenberg 2 FM_{eq}% (data not shown). An age effect was also found in most FM_{eq}% except for the Tyrrell, Shaefer and Deurenberg 1 FM_{eq}% in boys.

When analysing the mean difference, both over- and under-estimation occurred, although Tyrrell predominantly overestimated FM% in girls. Using regression analysis, best accuracy was reached with the Tanita® FM_{eq}% in girls as there was no difference from the line of identity (intercept=0 and slope=1). Furthermore, no significantly different slope could be detected for the FM_{eq}% of Slaughter and Deurenberg 1 in girls. Precision was best (high R^2 and low SEE) in Slaughter and Deurenberg 3, while worst in Deurenberg 1 and 2.

Furthermore, Bland-Altman analysis was used to analyse agreement on individual level. Agreement was highest (=small 95%LOA) in Slaughter and secondly in Deurenberg 3, while lowest in Deurenberg 2. Only the Deurenberg 2 for both sexes and the Tyrrell and Dezenberg for boys had no direction trend across the range of fatness after adjusting for age. Most other FM_{eq}% gave an upwards trend with more FM% underestimation in heavier children, although an overall downwards trend was present for the Schaefer FM_{eq}%. As a convergent dispersion trend was seen in half of the FM_{eq}%, the agreement with FM_{ADP}% increases with increasing FM%. Nevertheless, the Slaughter FM_{eq}% in boys had a divergent dispersion trend.

Highest interchangeability (high ICC) was seen in the Tanita® FM_{eq}% and secondly in Deurenberg 3 FM_{eq}%. On the other hand, overall lowest interchangeability was observed for the Deurenberg 2 FM_{eq}%. Nevertheless, in neither occasion the 0.75 cut-off to gain excellent interchangeability was reached.

When comparing the age-adjusted correlation coefficients of Table 19 and Table 20, the 'weight minus impedance index' obtained lower results than those of the FM_{eq}% based on impedance and approximately the same coefficients were retrieved for the sum of both skinfold thicknesses and both the Slaughter and Deurenberg 3 FM_{eq}%.

Table 20: Comparison between FMADP% and FMeq%, stratified by sex

	Difference (mean+/-SD; p-value) [a]	Age-adjusted correlation	Regression analysis: Accuracy Intercept	Regression analysis: Accuracy Slope	Regression analysis: Precision R^2	Regression analysis: Precision SEE	Age effect	Bland-Altman: Agreement 95%LOA with ranking [b]		Bland-Altman: Agreement Direction trend	Bland-Altman: Agreement Dispersion trend	ICC: Interchangeability
Tanita®												
male	0.9+/-4.1; .001	0.619*	3.324**	0.856†	0.425	4.01695	Yes	[-7.3;9.0]	4	Upwards‡	Convergent	0.619
female	2.2+/-3.8; <.001	0.749*	0.643	1.075	0.660	3.82922	Yes	[-5.5;9.8]	3	Upwards‡	Convergent	0.732
Tyrrell												
male	-5.4+/-4.2; <.001	0.601*	1.897**	0.688†	0.450	3.92085	No	[-13.9;3.1]	5	No	Convergent	0.438
female	-8.2+/-4.2; <.001	0.724*	-5.096**	0.897†	0.586	4.21652	Yes	[-16.7;0.3]	5	Upwards‡	Convergent	0.396
Shaefer												
male	5.6+/-5.5; <.001	0.563*	12.130**	0.480†	0.406	4.08658	No	[-5.4;16.5]	8	Downwards‡	Convergent	0.438
female	4.5+/-4.8; <.001	0.712*	10.309**	0.674†	0.600	4.15725	Yes	[-5.1;14.2]	7	Downwards‡	Convergent	0.638
Deurenberg 1												
male	0.3+/-4.6; .342	0.505*	5.984**	0.679†	0.313	4.39074	No	[-8.9;9.5]	6	Upwards‡	Convergent	0.550
female	-1.5+/-5.4; <.001	0.550*	1.507**	0.874	0.325	5.39228	Yes	[-12.2;9.3]	8	Upwards‡	No	0.505
Deurenberg 2												
male	-3.9+/-6.3; <.001	0.479*	9.703**	0.381†	0.246	4.60017	Yes	[-16.4;8.7]	9	Downwards	No	0.401
female	-4.5+/-6.6; <.001	0.537*	8.938**	0.499†	0.244	5.70865	Yes	[-17.6;8.7]	9	No	No	0.401
Slaughter												
male	3.6+/-3.5; <.001	0.706*	3.397**	1.017	0.551	3.55070	Yes	[-3.4;10.7]	1	Upwards‡	Divergent	0.541
female	4.6+/-3.4; <.001	0.800*	3.704**	1.052	0.735	3.38158	Yes	[-2.2;11.4]	1	Upwards‡	No	0.647
Goran												
male	2.3+/-4.0; <.001	0.675*	-4.605**	1.435†	0.479	3.82544	Yes	[-5.7;10.2]	3	Upwards‡	No	0.472
female	4.0+/-4.0; <.001	0.785*	-1.723**	1.314†	0.683	3.69928	Yes	[-3.8;11.8]	4	Upwards‡	No	0.588
Dezenberg												
male	1.4+/-5.4; <.001	0.567*	10.023**	0.481†	0.213	4.69960	Yes	[-9.4;12.1]	7	No	Convergent	0.447
female	0.3+/-4.8; .384	0.725*	4.068**	0.827†	0.498	4.65235	Yes	[-9.2;9.8]	6	Upwards‡	Convergent	0.697
Deurenberg 3												
male	1.9+/-3.7; <.001	0.707*	-4.270**	1.385†	0.552	3.53795	Yes	[-5.5;9.3]	2	Upwards‡	No	0.563
female	2.3+/-3.7; <.001	0.800*	-4.786**	1.352†	0.728	3.42286	Yes	[-5.2;9.7]	2	Upwards‡	No	0.710

*FMADP%= fat mass percentage with air-displacement plethysmography; ICC= intraclass correlation; SEE= standard error of estimate; LOA= limits of agreement; [a] FMADP% - FMEq%, paired t-test with Bonferroni correction; [b] ranking on interval width stratified by sex; *correlation with significant p-value; **Significantly different from 0; † Significantly different from 1; ‡ Maintained significance after correction for age*

Discussion

In this study sample of children between 5 and 11 years old, girls had higher adiposity and most anthropometric measurements were dependent on the child's age as their body is still growing. In contrast, no age differences in FM_{ADP}% were seen.

Skinfold thicknesses showed the best correlation with FM_{ADP}%. As indicated in literature, the age-adjusted correlations were highest for the sum of TSF and SSF, followed by the TSF (Freedman, Wang et al. 2007). This is also reflected by the frequent use of sum of skinfold thicknesses in published equations (see Table 17). If skinfold measurements are not feasible, the 'weight minus impedance index' can be used. As the impedance index is inherently a fat-free mass marker, the 'weight minus impedance index' resulted in higher correlations with FM_{ADP}% than the impedance index itself. Indeed, skinfold thickness is preferred above impedance as skinfold measurements are a more direct way of measuring FM.

Indices combining different measurements are not necessarily preferred. First, the ConI was one of the worst performing indices, as was also shown in previous research (Candido, Freitas et al. 2011). Second, the combined AFA index did not show a better correlation compared to the raw TSF (Chomtho, Fewtrell et al. 2006) and the WHtR was only slightly better than the waist circumference. The combined index WHR was considerably inferior to the WHtR as literature showed WHR is less suitable in measuring abdominal and total fat, also in children (Corvalan, Uauy et al. 2010). Still, we need to keep in mind that waist, WHR, WHtR and ConI are specifically reflecting central adiposity and therefore correlate less with total adiposity (Taylor, Jones et al. 2000). Overall, our findings are the first to replicate these literature findings (Candido, Freitas et al. 2011) in our Belgian childhood population, i.e. higher correlations in girls because of their higher prevalence of adiposity and best correlations for the sum of skinfold thicknesses.

When validating the FM% equations, our results showed that for BIA, the Tanita® FM_{eq}% was the best performing FM_{eq}%. There was no deviation from the line of identity in girls and it produced the best interchangeability, a small agreement interval and medium precision. A similar study comparing Tanita® FM_{eq}% with FM_{ADP}% also concluded the non-interchangeability with overall underestimation and an upward direction trend (Azcona, Koek et al. 2006). This non-interchangeability on individual level was published also for obese children with the four-compartment model due to substantial 95%LOA, even though there was no mean difference (Radley, Cooke et al. 2009). The Tyrrell BIA FM_{eq}% performed somewhat lower and was the only

one with an overall overestimation, confirming what was mentioned in their validation paper (Tyrrell, Richards et al. 2001).

For skinfold thickness, the Slaughter and Deurenberg 3 FM_{eq}% performed best in both boys and girls with high precision (high R^2 and low SEE) and good agreement on individual level (small 95%LOA interval). Our skinfold equation results are quite precise as the SEE values in Table 20 are in the same range as the original published Deurenberg 3 (between 3 and 5%) and Slaughter (3.7%) SEE values (Slaughter, Lohman et al. 1988). Nevertheless, both the Slaughter and Deurenberg 3 resulted in an overall FM% underestimation. Previously, the Slaughter equation was successfully cross-validated with underwater weighing by Janz et al with also better results for girls and SEE values similar to ours (Janz, Nielsen et al. 1993). Nevertheless, overall FM% overestimation was shown by Paineau when using a three-compartment model with similar 95%LOA as ours (Paineau, Chiheb et al. 2008), while FM% overestimation in boys and underestimation in girls was shown using underwater weighing with the same direction trend as observed in our study (Reilly, Wilson et al. 1995). In the latter study from Reilly, the Slaughter equation showed the best accuracy. The Deurenberg 3 FM_{eq}% has previously shown a similar agreement interval as ours when comparing with the three-compartment model, although it resulted in an overall overestimation (Paineau, Chiheb et al. 2008). In the same study, the Deurenberg 3 FM_{eq}% based on four skinfolds performed best and the Slaughter FM_{eq}% had a wider agreement interval than the Deurenberg 3 FM_{eq}%. Indeed, also in our study the Deurenberg 3 skinfold equation could be preferred above the Slaughter skinfold equation because of the better interchangeability and bias on population level. Other articles cross-validating the Deurenberg 3 based on only TSF and SSF were not found since most researchers used the four skinfold equation.

In conclusion, the Deurenberg 3 equation was the best skinfold equation (although inferior to the index ‘sum of skinfold thicknesses’) and the Tanita® equation was the best BIA equation (even better than the ‘weight minus impedance index’). Furthermore, Dezenberg was the worst performing FM_{eq}% based on skinfold thickness, while the Deurenberg 2 FM_{eq}% based on impedance was the worst performing overall.

When creating a new equation, a certain methodology is performed in a selected population sample. The characteristics of the methodology and the population sample are inherent restrictions to the usefulness of this equation in other studies. First, we will consider the methodology of published equations. For BIA, only equations using the impedance (as we have measured), and not the resistance or reactance were selected. As only the Tanita® and Tyrrell equations were validated for

foot-to-foot BIA, also some whole-body equations were tested. For the skinfold equations, both TSF and SSF are normally included, although the Dezenberg equation was restricted to only TSF. DEXA and underwater weighing are often taken as reference method, although the four-compartment model is the only golden standard. Secondly, it is crucial to consider the population in which the equation was validated. We only selected equations validated in at least a child population. The Tyrrell, Deurenberg 2 and Dezenberg equations were validated in a population with a much higher FM% than ours, while Tyrrell, Slaughter and Dezenberg were validated in non-European groups and the Deurenberg and Slaughter equations did not fully cover our age range.

Some of these population and device characteristics (Table 17) can partially explain the performance of the FM_{eq}% in our population (Table 20). Obviously, the Tanita® equation had the best BIA FM_{eq}%. This can have several reasons: (1) it was validated specifically for the device, (2) our age range was fully covered and (3) age, sex and weight were considered. Nevertheless, the original Tanita® validation used another reference technique than ours and population characteristics of their validation study are not available. The Tyrrell equation might be somewhat lower ranked due to its validation population (much higher FM%, also non-European) and another type of the BIA device. As expected, the FM_{eq}% based on whole-body BIA were less appropriate when using data from foot-to-foot BIA, although no general trend in over- or under-estimation was seen. Especially the Deurenberg 2 FM_{eq}% did not fit at all as its validation population was far more obese and did not totally cover our age range. In the skinfold equations, Dezenberg FM_{eq}% was the worst probably due to the exclusive use of triceps skinfold thickness (while the sum of skinfold thicknesses performed best in our analysis) and the population characteristics (mixed population and high obesity level).

As such, caution is needed when using equations as the prediction equation may have a profound effect on the FM% estimate. Biological (age, FM%, ethnicity) and methodological (foot-to-foot versus whole-body BIA, trained staff, reference method) factors should be considered in choosing a published equation. For example, the Dezenberg equation performed differently between US and UK children (Wells 1999). Furthermore, researchers should be aware that the magnitude (dispersion trend) and direction (direction trend) of the error is depending on the FM% of the studied children. Often, we have seen a convergent dispersion trend in parallel with what has been stated in literature: better FM% prediction agreement with increasing FM% (Bray, DeLany et al. 2001). Overall, the use of prediction equations inevitably confounds the raw measurements with prediction error, even if a similar population is used. We have shown that even the best skinfold equation did not perform better than the sum of skinfolds.

It is noteworthy that none of the best-performing equations (Tanita® and Deurenberg 3) is perfect for overweight detection as they both resulted in overall underestimation, especially in the high FM% area (an upward direction trend). This direction trend through the FM% range is in accordance with previous findings of Reilly et al. (Reilly, Wilson et al. 1995). They showed no excellent interchangeability with FM_{ADP}% and quite broad 95%LOA intervals: approximately 14, 15 and 16%FM for Slaughter, Deurenberg 3 and Tanita®, respectively, while the maximum range of FM_{ADP}% was on average 33%FM. Indeed, agreement intervals of 16 and 18% were previously shown (Wells and Fewtrell 2006). This indicates that results obtained with these equations should be interpreted with caution on the individual level. Furthermore, the non-interchangeability (based on ICC) justifies the use of an immobile device like the BOD POD® despite the higher workload and cost and more complicated survey management.

Strengths and limitations

One of the major strengths of this paper is the large-scale comparison of equations based on BIA or skinfolds and anthropometric data and indices with ADP, as such a large-scale use of ADP is not always feasible. Consequently, we could formulate recommendations for anthropometric measurements in future field studies with Caucasian children. Furthermore, we used elaborated statistics with analyses stratified by sex as sex differences were noticed. As such, Bland-Altman trend and age effects were given independently for sex. In addition, age correction was done for the Bland-Altman trend. Finally, the routine anthropometric measurements were highly standardized and conducted by two trained researchers to minimize inter- and intra-observer variability.

Nevertheless, some limitations remain. We could not use the highly recommended four-compartment model as reference. As we have stated in the introduction, this is almost not feasible in such a large child population due to time, budgetary and participation-rate constraints. Consequently, air-displacement plethysmography was used as criterion method. Nevertheless, the results of this validity study can be confounded by some methodological aspects. First of all, BOD POD® and anthropometrics were not systematically measured on the same day as BOD POD® measurements were optional and were not performed at school where the field work took place, although it was tried to make the appointment in the same week. This could have biased the differences found between the methods for some individuals. Second, thoracic gas volume was estimated rather than measured. Although a child-specific formula was used, measuring it with the breathing tubes delivered with the device will lead to higher accuracy of the measurement. In the

validation of the child-specific equation, no significant difference was found with the measured gas volume and a low SEE (0.369) was found, even though this could result in a FM% error up to 2% fat units (Fields, Hull et al. 2004). Nevertheless, this protocol is difficult and time-consuming (because of failure) and could decrease participation rate by anxiety feelings in children. In a previous study, 75% of all children and adolescents needed at least three trials for estimation (Fields, Hull et al. 2004). Third, the ratio of chamber volume to subject volume is quite large in children and as such, the BOD POD error might be greater in the smaller than in the older children (Fields and Goran 2000). Another limitation is that the selection of published equations was restricted by measuring only two skinfolds and by using the foot-to-foot BIA, as many equations use four skinfolds or are based on whole-body BIA. However, the foot-to-foot BIA device is being increasingly used on the field due to its simplicity and a recent review claimed its non-inferiority to the more complicated whole body BIA devices (Jaffrin 2009). Finally, our relatively low FM% range (6.7% overweight) restricts the generalization of our results to populations with higher FM% and restricts the detection of a Bland-Altman trend across higher FM%.

Conclusion

Our results support the use of the sum of triceps and subscapular skinfold thicknesses as marker of FM% in Caucasian children when specialized technology like ADP is not feasible. Nevertheless, trained staff is needed for skinfold measurements to reduce inter- and intra-observer variability. Although the Deurenberg gave the best results of all skinfold equations, the use of equations should be limited for several reasons: (1) no excellent interchangeability with FM_{ADP}% could be detected; (2) equations validated in a different population or with slightly different methodology will result in higher prediction errors; (3) the magnitude and direction of the error is dependent on the FM%, age and sex of the population. If skinfolds are not feasible due to untrained staff, BIA with the built-in Tanita® equation can be used in Caucasian children. Furthermore, BMI, arm circumference, waist-to-height ratio and 'weight minus impedance index' can be used as parameters for FM% of only intermediary quality. Nevertheless, the higher workload, cost and more complicated survey management of an immobile device like the BOD POD remains justified in large-scale child studies.

IV.

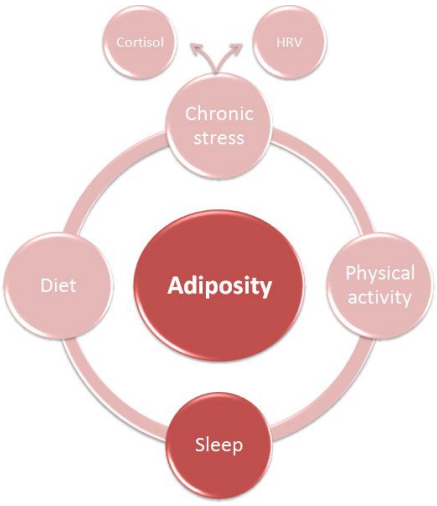
LIFESTYLE RESULTS

1.

Children's sleep quality: relation with sleep duration and adiposity

Michels N, Verbeiren A, Ahrens W, De Henauw S, Sioen I

Public Health, short communication in review.



The only thing people like

that is good for them:

a good night's sleep.

ABSTRACT

OBJECTIVES: Low sleep duration has been linked with overweight, but little research has considered the aspect of sleep quality, especially not in children.

STUDY DESIGN: Cohort study in 6-12y old Belgian children measured in 2010 (N=239) and 2012 (N=193).

METHODS: Sleep duration was reported by the parents and actigraphy was used to measure sleep quality (sleep efficiency, sleep latency, nocturnal awakenings). Markers of adiposity were determined: body mass index (overall), fat percentage (overall) and waist circumference (central). Linear regressions examined the relation of sleep quality with sleep duration and the relation of both sleep quality and duration with adiposity.

RESULTS: Sleep duration and sleep quality were associated: children with a longer reported sleep duration had a longer actual sleep duration but not in proportion with their extra time spent in bed since they had a lower sleep quality (due to longer sleep latency and more awakenings). Sleep duration - but not sleep quality - was cross-sectionally and longitudinally associated with higher adiposity (both central and overall).

CONCLUSIONS: Results confirmed the unfavourable association of short sleep duration - but not sleep quality - with body composition. This highlights the importance of sleep in overweight prevention. Nevertheless, long sleep duration does not assure a better sleep quality, even the contrary was shown. The effect of sleep quality should be further examined longitudinally in a more heterogeneous population sample, with correction for sleep duration and additionally studying changes in sleep architecture and hormones.

Introduction

A trend towards insufficient sleep duration and increasing sleep problems has been reported in children (Mindell and Owens 2003). Several reviews have linked short sleep duration with adiposity, showing the strongest association in children and young adults (Knutson and Cauter 2008, Patel and Hu 2008). Apart from sleep duration (hours spent in bed), sleep quality (i.e. an undisturbed, efficient sleep) should not be ignored. When having problems falling asleep or when suffering from frequent awakenings, actual sleep duration decreases and the body will not spend enough time in critical sleep phases, even if the total sleep duration remains sufficient (Mindell and Owens 2003). Mostly, sleep quality has been measured by questionnaire (questions on problems falling asleep and frequent awakenings), but sleep quality can also be measured objectively with actigraphy (sleep efficiency by measuring movements during sleep) or polysomnography (measuring sleep architecture). Limited research has linked this qualitative aspect of sleep with adiposity and most of the studies were based on subjective reports or focused on adults (Beccuti and Pannain 2011). The scarce literature in children is solely based on cross-sectional studies; most of them found a negative association between sleep quality and adiposity (Gupta, Mueller et al. 2002, Landis and Parker 2007, Liu, Forbes et al. 2008, Bawazeer, Al-Daghri et al. 2009, Liu, Hay et al. 2011), although also a non-significant result has been published (Mota and Vale 2010). A main hypothesis for an effect of sleep quality on adiposity is that low sleep quality not only decreases actual sleep duration, but that sleep quality in itself could also induce hormonal changes (more cortisol and less growth hormone secretion) due to alterations in the sleep architecture. These hormonal changes finally stimulate increased fat deposition, especially in the visceral regions (Van Cauter, Leproult et al. 2000). Consequently, sleep quality might be linked with body composition. Therefore, we aimed to test (1) the relation between sleep duration and sleep quality as separate concepts and (2) the independent effects of low sleep duration and low sleep quality on adiposity in children. Since subjective reports of sleep quality do not correlate well with objective measurements, we used an objective sleep quality measure in contrast to the often used questionnaires. Since the sleep-adiposity relation could be bidirectional, longitudinal data allows us to examine the unidirectional effect of sleep on adiposity. Moreover, adiposity was measured in a standardized way reflecting both overall and central obesity.

Methods

For this study, sleep quality was measured in a subpopulation of the 523 children (6-12y old, 52% boys) that participated in the Belgian longitudinal ChiBS (Children's Body composition and Stress) study (Michels, Vanaelst et al. 2012): 239 children had data in 2010 of which 193 children had follow-up data in 2012. The children without sleep quality data (N=284) did not differ in adiposity, socio-economic status, age or sex compared to the children included in the analyses.

Children's sleep was reported by the parents using a sleep diary and it was measured objectively by actigraphy at the wrist (Actigraph®, Pensacola, FL, USA). Actigraphy data was processed with Actilife-software using the Sadeh scoring algorithm (Sadeh, Sharkey et al. 1994). At least four nights including both week and weekend days (between 4 and 7 nights with a mean of 5) and a complete sleep diary were necessary for interpretation. Sleep duration was represented by 'reported sleep duration': the time between reported 'time in bed' and 'time out bed'. The following four sleep parameters were obtained to represent sleep quality: (1) sleep latency or time between 'time in bed' and sleep onset, (2) minutes scored as wake after sleep onset (WASO), (3) actual sleep duration or corrected sleep duration without sleep latency and WASO, (4) sleep efficiency defined as 'the proportion of actual sleep over the total time spent in bed'. The sleep quality data showed good stability since the intra-class correlation coefficient ranged between 0.690 and 0.791. To correct for week/weekend differences in further analyses, all sleep data was weighted as follows: 2/7*weekend and 5/7*week. Including a variable on oversleeping during the weekends (the difference between week and weekend reported sleep duration) did not alter the relation between quantity-quality and sleep-adiposity.

The cross-sectional and longitudinal effect (over two years) of the reported sleep duration and measured sleep quality (independent of duration) on adiposity was explored. Adiposity was not only represented by the classical BMI parameter using age- and sex- specific BMI z-scores according Cole's method (Cole, Freeman et al. 1998), but also by using the more advanced fat% by air-displacement plethysmography (BOD POD® device) and by using waist circumference to reflect central fat deposition. The analyses were adjusted for age, sex, parental education, physical activity (actigraphy measurements using the same device but located at the hip during the day) and weekly snacking frequency (reported in a food frequency questionnaire).

Results and discussion

On average, a healthy body weight was seen with only 7% of the children being overweight/obese using the International Obesity Task Force classification of children's BMI. Considering a recommended sleep duration of 10 to 11 hours for children in this age category (National Sleep Foundation), only 14.2% had less sleep than recommended, 61.7% had the recommended amount of sleep, and 24.1% had a longer sleep duration, based on the reported sleep duration. These low adiposity and sleep lack numbers will limit the generalizability of our results to the overall population. Means and standard deviations are shown in Table 21.

A first aim was to examine the relation of sleep quality with reported sleep duration. Linear regression with adjustment for age and sex showed highly significant associations between reported sleep duration and sleep quality parameters ($p<0.001$). This was found in both younger and older children and for both weekdays and weekend days. However, with a reported sleep duration (=being in bed) of 1 hour longer, the actual sleep duration was only 24 minutes longer as they had a 5 minutes longer sleep latency, 29 minutes more WASO and therefore a 3% lower sleep efficiency. Consequently, children with a longer reported sleep duration may have a longer actual sleep duration but not in proportion with their extra time spent in bed since they have a lower sleep quality. A negative relation between reported sleep duration and sleep quality makes sense when considering our small percentage of children sleeping less than recommended (14.2%): those with a more than sufficient reported sleep duration might not be sleepy anymore and might have more awakenings or a longer sleep latency. An intervention study illustrated this idea as a one-hour sleep restriction could induce higher sleep quality (less awakenings and higher efficiency) in children compared to a one-hour sleep extension (Sadeh, Gruber et al. 2003). Nevertheless, children's WASO was in another study negatively and sleep efficiency positively correlated with reported sleep duration (Buckhalt, El-Sheikh et al. 2007).

A second aim was to examine the effect of sleep (duration and quality) on adiposity (three separate parameters: BMI, fat% and waist). This was done using cross-sectional SPSS linear regression (baseline associations in 2010) and longitudinal SPSS mixed models linear regression (change over 2 years based on the two measurements in 2010 and 2012, respectively). Table 21 shows the longitudinal regression analyses for the effect of sleep duration and quality on adiposity parameters. Cross-sectional analyses showed the same pattern. No sex differences were found. Reported sleep duration was negatively associated

with all three adiposity measures and actual sleep duration was negatively associated with waist circumference (although fat% and BMI also showed borderline significance). No associations were seen between other sleep quality parameters (sleep efficiency, sleep latency and WASO) and adiposity. Results remained the same after correction for reported sleep duration (i.e. there is also no independent effect of sleep quality) and lifestyle (physical activity and snacking). The associations for reported sleep duration became even more significant after correction for sleep quality. Low sleep quality values may not have deleterious effects on adiposity in our population simply because most children have a long sleep duration (mean=10.6h) which may keep the actual sleep duration high. Nevertheless, actual sleep duration had an effect on waist change over a period of two years, independently from reported sleep duration.

Table 21: Sleep duration and quality as predictors for adiposity evolution between first (2010) and second (2012) measurement

		body mass index (N=193)		**fat% (N=132)**		**waist (N=193)**	
	Mean (SD)	15.9 (1.9)		19.5 (6.3) %		57.1 (6.1) cm	
		B	**p**	**B**	**p**	**B**	**p**
Reported data =sleep quantity							
reported sleep duration	10.6 (0.6) h	-0.381	0.030*	-2.348	0.002*	-1.666	0.016*
Accelerometer data = sleep quality							
actual sleep duration	8.3 (0.7) h	-0.230	0.090	-1.082	0.067	-1.083	0.014*
sleep latency	20.3 (13.5) min	-0.001	0.830	0.002	0.940	-0.011	0.677
wake after sleep onset	110 (37.0) min	0.001	0.868	-0.004	0.715	0.003	0.744
sleep efficiency	78.9 (5.9) %	-0.008	0.589	-0.004	0.956	-0.044	0.465

**p<0.05; B= unstandardized coefficient. Longitudinal linear mixed regression corrected for age, sex, parental education, physical activity and, snacking frequency. Accelerometer data was additionally corrected for reported sleep duration. Same parameters were significant without correction for physical activity, snacking and sleep duration.*

Conclusion

In summary, analyses on our first aim have shown that long sleep duration does not guarantee a higher sleep quality or efficiency, even in the contrary. Since sleep duration and quality showed an inverse relation in our population, future studies should also correct for sleep

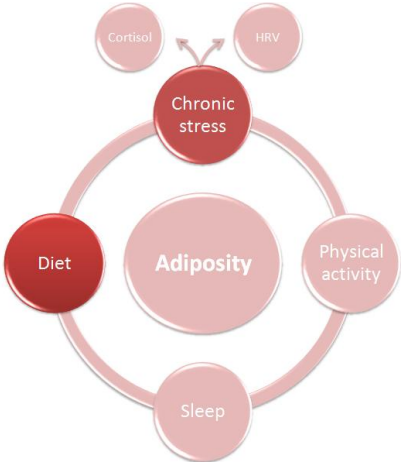
duration to examine the independent effect of sleep quality (as demonstrated in this study). In testing our second aim, we have confirmed the unfavourable effect of short sleep duration (sleep quantity) on overall and central adiposity. In contrast to the main literature, we could not confirm the unfavourable effect of objective low sleep quality (except for an unfavourable effect of low actual sleep duration on central adiposity). An important underlying reason might be that previous cross-sectional reports (with a higher overweight prevalence) reflected mainly an effect of adiposity on sleep quality instead of an effect of sleep quality on adiposity. This might highlight the importance of longitudinal and experimental designs in studying directionality. Nevertheless, our data cannot be generalized to the overall population due to low overweight percentages, quite good sleep duration and the young age group. Also, sleep staging (by using polysomnography instead of actigraphy) and hormonal analyses in large population samples should be stimulated to further elucidate the possible relation. Remarkably, the effects of actual sleep duration were mainly found on the central adiposity measure. This inspires future studies to consider several markers of adiposity.

2.

Stress, emotional eating behaviour and dietary patterns in children.

Michels N, Sioen I, Braet C, Eiben G, Hebestreit A, Huybrechts I, Vanaelst B, Vyncke K, De Henauw S

Appetite, 2012: 59(3): 762-9.



Food was the glue

that kept me together.

ABSTRACT

Psychological stress has been suggested to change dietary pattern towards more unhealthy choices and as such to contribute to overweight. Emotional eating behaviour could be an underlying mediating mechanism. The interrelationship between stress, emotional eating behaviour and dietary patterns has only rarely been examined in young children. Nevertheless, research in children is pivotal as the foundations of dietary habits are established starting from childhood and may track into adulthood. In 437 children (5-12y) of the ChiBS study, stress was measured by questionnaires on stressful events, emotions (happy, angry, sad, anxious) and problems (emotional, peer, conduct and hyperactivity). Data were collected on children's emotional eating behaviour and also on dietary patterns: frequency of fatty foods, sweet foods, snacks (fat and sweet), fruit and vegetables. Stressful events, negative emotions and problems were positively associated with emotional eating. Positive associations were observed between problems and both sweet and fatty foods consumption. Negative associations were observed between events and fruit and vegetables consumption. Overall, stress was associated with emotional eating and a more unhealthy dietary pattern and could thus contribute to the development of overweight, also in children. Nevertheless, emotional eating behaviour was not observed to mediate the stress - diet relation.

Introduction

The importance of a healthy diet is widely accepted. More specifically, dietary guidelines are formulated in the prevention of obesity with a focus on high intakes of fruit and vegetables and low intake of energy dense foods like those high in fat and sugar (WHO 2003). The foundations of dietary habits are established from the ages of 3-4 years old (Singer, Moore et al. 1995) and may track into adolescence and adulthood (Wang, Bentley et al. 2002, Mikkila, Rasanen et al. 2005).

An overall healthy diet consists of both a balanced food and nutrient composition as well as a balanced eating behaviour. A balanced eating behaviour comprises eating when feeling hungry, at regular moments to allow physiological growth and energy expenditure. However, a trend of eating in the absence of hunger and intermittent snacking is increasingly observed in the eating pattern in Western society. This unhealthy eating behaviour, is related with unfavourable outcomes (unbalanced intake with too much fat and sugar leading to overweight in genetic at risk groups), making it pivotal to study its determinants.

Stress has been associated both with unhealthy emotional eating behaviour and an imbalanced dietary pattern (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Macht 2008). However, several research gaps remain unresolved. For example it is relevant to test if stress is related with specific indices of food intake like higher consumption of fatty foods, sweet foods or both (e.g. snacks) and whether emotional eating can be seen as the mechanism underlying the assumed link between stress and an imbalanced dietary pattern. After all, emotional eating is hypothesized as a way of avoidant stress coping, eating induced stress reduction or because of the reward feelings associated with the food (Dallman, Pecoraro et al. 2003, Adam and Epel 2007).

Epidemiological research investigating the influence of stress on children's diet is rather scarce and has mostly been performed in small samples, among adolescents, in laboratory conditions or focused on only one aspect of stress and mostly with indirect measures of imbalanced eating (e.g. increase in weight). As far as we know, only one study in children has included both a specific naturalistic stress measure and a direct measure of the individual's dietary pattern (Jenkins, Rew et al. 2005).

Therefore, this study aims to fill this research gap by investigating the relationship between several stress measures, emotional eating behaviour and dietary patterns (sweet foods, fatty foods, snacks but also fruit and vegetables) in a sample of preadolescent children. Furthermore, the possible mediation effect of emotional eating behaviour will be tested.

Especially in young children studying the role of stress on dietary intake is challenging as parents still highly control their child's diet, while at the same time children already start to develop their own preferences.

Methods

Participants and general procedures

The subjects were Belgian children (49.9% boys) between 5 and 12 years old participating in the ChiBS study in 2011 (first follow-up). In 2011, 453 children participated in the survey but 16 questionnaires were incomplete. As a result, analyses for this paper were based on a sample of 437 children. Detailed aims, design, methods, population and participation characteristics are described in chapter 2 "Methodology".

Questionnaires on children's stress: problems, emotions, life events and coping

Stress arises when the demands of a situation exceed an individual's ability to cope and resolve the problem, resulting in emotional, behavioural and cognitive disturbances (McCance, Forshee et al. 2006). Besides life events, which are assumed to profoundly affect someone's life, daily annoyances and minor hassles are also seen as an important stressor (Kanner, Coyne et al. 1981). However, some events can have greater impact than others. Therefore, stress is for research purposes often operationalized on the symptom level by measuring (1) the daily problems that someone is reporting or (2) the daily emotions someone is feeling or (3) the way someone is handling the problems and feelings.

Life events (child-reported)

The Coddington Life Events Scale for children (CLES-C) assesses the frequency and timing of 36 stressful life events relevant for this age group during the last year (see chapter 2 "Methodology"). For this paper, the negative life events score was calculated for the previous 6 months.

Daily events: hassles and uplifts (child-reported)

The children's daily hassles (CHS) and daily uplifts (CUS) scales (Kanner, Coyne et al. 1981) enquire the occurrence and intensity of 25 hassles and 25 uplifts during the last month. More details can be found in chapter 2 "Methodology". An intensity score was used for the hassles

(=severity sum of the indicated events) and a frequency score for the uplifts (=amount of events).

Emotions (child-reported)

Children were asked to report on their recent feelings of anger, anxiety, sadness and happiness as described in chapter 2 "Methodology".

Coping (child-reported)

The children were asked what they usually do when confronted with problems or when they are upset using an 8 item-questionnaire. Validation data can be found in chapter 2 "Methodology". The answers were classified as emotion- versus problem-focused coping, based on the transactional model of Lazarus and Folkman (Folkman and Lazarus 1986). A coping index was calculated as "problem-focused coping minus emotion-focused coping", with positive values representing more problem-focused coping.

Problems (parent-reported)

Parents were asked to complete the 'Strengths and Difficulties Questionnaire' (SDQ) (Coddington 1972, Coddington 1999), reporting the problems of their child over the past 6 months. The 25 statements were divided in 5 subscales of 5 items each: emotional problems, conduct problems, hyperactivity problems, peer problems and prosocial behaviour. Higher scores on the prosocial behaviour subscale reflect strengths, whereas higher scores on the other four subscales reflect difficulties or problems.

Questionnaires on children's emotional eating behaviour and dietary patterns

Emotional eating behaviour (child-reported)

In the Dutch Eating Behaviour Questionnaire (DEBQ) three types of maladaptive eating behaviour can be identified (van Strien, Frijters et al. 1986). More information can be found in chapter 2 "Methodology". For this study only emotional eating (eating in response to negative emotions) will be considered.

Dietary patterns (parent-reported)

The Food Frequency Questionnaire (FFQ) is a screening instrument to investigate food consumption frequency of 43 food items. To identify dietary patterns, four 'food indices' on

dietary pattern were calculated by summing up the frequency of separate food items: a food index for 'sweet foods' (sweet drinks, jam, honey, sweet breakfast cereals, sweet snacks), 'fatty foods' (fried potatoes, chocolate- or nut-based spreads, high fat dairy, mayonnaise and mayonnaise-based products, cheese, fat meat preparations, butter, high fat snacks), 'snacks' (chocolate and chocolate bars, candy, biscuits, cake, ice-cream, chips, savoury pastries) and also a healthy food index for 'fruit and vegetables' (fruit, freshly squeezed fruit juice, vegetables) was used.

Confounders

Parental education (as marker for socio-economic status) and children's body fat percentage were measured as described in chapter 2 "Methodology".

Statistical methods

Analyses were done using PASW Statistical Program version 19.0 (SPSS Inc, IBM, IL, USA). The two-sided level of significance was set at $p<0.05$.

To reduce multicollinearity and the amount of analyses, principal component analysis on the stress questionnaires (problems, emotions, life events, daily events and coping) was done using promax rotation. The Keiser-Meyer-Olkin Measure and the significant Barlett's test of sphericity suggested that the data were amenable for this analysis. The stress questionnaire data was transformed in z-scores before inclusion in the analysis. Based on the screeplot, a three factor structure was chosen. These three factors will be used in all further analyses instead of the separate questionnaire responses.

The association between stress and emotional eating behaviour and dietary patterns (by four food indices) was analysed using Spearman correlations. A mediation model for emotional eating in the relation stress versus dietary patterns was tested. A first set of regression analyses was done using the food index as dependent variable (either frequency of sweet foods, fatty foods, snacks or fruit and vegetables). A second set of analyses tried to predict emotional eating by stress factors. Mutual interactions were checked. Analyses were corrected for age, sex, socioeconomic status and fat%. According to Baron and Kenny (1986), mediation is present when the following conditions are met: (1) the independent variable (stress factors) significantly predicts the mediator (emotional eating), (2) the independent variable (stress factors) significantly predicts the dependent variable (food indices), (3) the mediator (emotional eating) significantly predicts the dependent variable (food indices) and

(4) the relationship between the independent variable (stress factors) and the dependent variable (food indices) should be significantly reduced after controlling for the mediator (emotional eating). The significance of this fourth (and indirect) effect was tested using the Sobel test (Sobel 1982). Furthermore, this indirect effect was also tested nonparametrically by bootstrapping (using 10000 samples) (Preacher and Hayes 2004).

Results

Descriptive data

As mentioned before, the principal component analysis resulted in a three factor structure for stressors. The first factor (called 'Problems') was positively affected by conduct problems, hyperactivity, peer problems and emotional problems and negatively by prosocial behaviour. The second factor (called 'Negative emotions') was positively affected by sadness, anger, anxiety and negatively by happiness. The third factor (called 'Events') was positively affected by daily uplifts, daily hassles and negative events during the last 6 months. These three factors will further be used as stress parameters.

Descriptive data on our child population is given in Table 22. Boys and girls were equally distributed in our sample. No sex differences were seen in emotional eating behaviour measured by the DEBQ or in dietary patterns measured by the FFQ. Boys reported more problems (hyperactivity problems and total problems) and negative life events the last 6 months, while they had lower scores on anxiety and prosocial behaviour.

Table 22: Descriptive statistics of the 437 participating children

	boys N=218		girls N=219		sex difference
	median	**IQR**	**median**	**IQR**	**(p)**
Confounders					
age (years)	8.96	7.80 - 10.09	9.02	7.79 - 9.99	0.606
fat percentage (%)	16.57	13.79 - 20.52	20.88	16.72 - 26.83	<0.001*
socioeconomic status [a] (%high)	55.07%		51.89%		0.557
Stress: raw questionnaire data					
Strengths and Difficulties scores					
emotional problems (0-10)	2	1 - 4	2	1 - 4	0.068
conduct problems (0-10)	1	0 - 2	1	0 - 2	0.083
hyperactive problems (0-10)	3	2 - 6	2	1 - 4	<0.001*
peer problems (0-10)	1	0 - 2	1	0 - 2	0.687
prosocial behaviour (0-10)	9	7 - 10	9	9 - 10	0.002*
Emotion scores					
happy (0-10)	8	6 - 9	8	6 - 10	0.637
angry (0-10)	2	1 - 3	2	1 - 4	0.962
sad (0-10)	2	0 - 4	2	0 - 4	0.495
anxious (0-10)	0	0 - 2	1	0 - 3	0.006*
Life and daily events					
negative event score last 6 months	34	0 - 80	0	0 - 56	0.014*
daily intense hassles (0-100)	42	28 - 54	41	27 - 55	0.735
daily uplifts (0-25)	23	19 - 24	22	19 - 24	0.500
Coping					
problem versus emotional coping (-10-10)	2	0 - 3.67	2	0.67 – 4.00	0.088
Stress: summarizing factors					
factor 1: Problems (z-score)	-0.07	-0.65 - 0.75	-0.29	-0.87 - 0.38	0.002*
factor 2: Negative emotions (z-score)	-0.29	-0.75 - 0.37	0.01	-0.74 - 0.86	0.051
factor 3: Events (z-score)	0.16	-0.44 - 0.72	0.01	-0.61 - 0.67	0.255
Eating behaviour (DEBQ)					
emotional eating (1-5)	1.92	1.38 - 2.50	1.92	1.46 - 2.38	0.811
Dietary pattern					
sweet food frequency (times/week)	29	22 - 39	27	20 - 36	0.277
fatty food frequency (times/week)	25	18 - 33	25	19 - 32	0.852
snack frequency (times/week)	9	6 - 14	9	6 - 13	0.473
fruit and vegetables frequency (times/week)	14	11 - 19	14	10 - 19	0.088

** p< 0.05; [a] based on maximal parental education: high= tertiary or higher education; DEBQ=Dutch Eating Behaviour Questionnaire; IQR= interquartile range*

Correlations

Table 23 shows the correlations between stress parameters, emotional eating behaviour and dietary patterns. The Problems and Events factor were positively correlated with emotional eating. Problem-focused coping was not associated with emotional eating. The sweet and fatty food indices were positively correlated with Problems and the fruit and vegetables food index negatively with Events.

Mediation of emotional eating behaviour in the stress – dietary patterns relation

Table 24 and Table 25 present the regression analyses for dietary patterns and emotional eating behaviour as dependent variables respectively. No interaction effect of stress and emotional eating behaviour was detected. Problems, Negative emotions and Events were positive predictors for emotional eating. These associations differed between boys and girls, with Problems being only significant in boys, Emotions only in girls and Events in both sexes. Children with more Problems had a higher sweet and fatty food index, while children with more Events had a lower fruit and vegetables food index. After correction for emotional eating behaviour, significances remained. Stratified by sex, these stress – dietary patterns relations remained significant for girls, but not for boys (data not shown). Yet, emotional eating behaviour was no significant predictor for dietary patterns, even not after stratification into 2 age groups (data not shown). Furthermore, no age interactions were seen for the analyses in Table 24 and Table 25.

Figure 23 illustrates the possible mediation models based on the regression analyses for which at least two of the four conditions were met. In all the models, stress (Problems and Events) was a significant predictor for dietary patterns. Furthermore, the path between stress (Problems and Events) and emotional eating was also significant. Nevertheless, no relationship was found between emotional eating and dietary patterns. Also, the Sobel test and the bootstrapping of the indirect effect indicated no mediation activity of self-reported emotional eating in the relationship between stress and dietary patterns.

Table 23: Correlations between stress parameters (both separate items and summarizing factors), emotional eating behaviour and dietary patterns

		stress parameters														
		strengths and difficulties questionnaire					emotions				life events	daily events		coping	summarizing factors out of principal components analysis		
		emotional problems	conduct problems	hyperactivity problems	peer problems	social behaviour	happy	angry	sad	anxious	6 month events	hassles	uplifts	coping index [a]	factor 1 Problems	factor 2 Emotions	factor 3 Events
emotional eating	r	**0.142****	0.089	**0.130****	-0.025	-0.042	-0.068	0.025	0.051	0.012	0.008	**0.292****	**0.104***	-0.014	**0.119***	0.094	**0.207****
dietary patterns: food consumption frequency																	
sweet foods	r	**0.107***	**0.108***	**0.151****	0.084	**-0.118***	-0.083	0.005	0.057	0.001	-0.025	0.033	-0.079	-0.078	**0.183****	0.054	0.002
fatty foods	r	**0.101***	0.068	0.034	0.065	**-0.104***	-0.069	-0.036	0.072	-0.010	-0.019	-0.037	**-0.122***	-0.090	**0.117***	0.049	-0.092
snacks	r	0.017	0.027	-0.010	-0.020	-0.085	-0.001	-0.044	-0.008	-0.039	-0.067	0.016	-0.084	-0.001	0.015	-0.054	-0.035
fruit and vegetables	r	0.053	-0.021	**-0.122***	0.008	0.062	0.055	0.020	-0.043	-0.016	-0.092	-0.055	-0.041	0.022	-0.023	-0.012	**-0.112***

*r= spearman correlation coefficient; * p <0.05; ** p < 0.01; [a] problem-focused coping minus emotion-focused coping*

Table 24: Regression analyses: effect of emotional eating behaviour and stress factors on dietary patterns (food indices as dependent variables)

	snacks			sweet foods			fatty foods			fruit and vegetables		
	beta	**t**	**p**	**beta**	**t**	**p**	**beta**	**t**	**p**	**beta**	**t**	**p**
emotional eating	0.011	0.217	0.828	-0.011	-0.216	0.829	0.035	0.712	0.477	-0.050	-1.018	0.310
factor 1: Problems	0.004	0.082	0.934	0.150	3.039	0.003*	0.115	2.308	0.022*	-0.045	-0.896	0.371
factor 2: Negative emotions	-0.026	-0.523	0.601	0.026	0.529	0.597	0.061	1.219	0.224	-0.026	-0.509	0.611
factor 3: Events	-0.048	-0.956	0.340	-0.011	-0.211	0.833	-0.086	-1.730	0.084	-0.106	-2.127	0.034*

** p<0.05; confounding variables (age, sex, socioeconomic status and fat percentage) have been taken into account in the statistical analysis*

Table 25: Regression analyses: effect of stress factors on emotional eating behaviour (emotional eating as dependent variable)

	emotional eating		
	beta	**t**	**p**
factor 1: Problems	0.114	2.249	0.025*
factor 2: Negative emotions	0.116	2.309	0.021*
factor 3: Events	0.204	4.146	<0.001*

** p<0.05; confounding variables (age, sex, socioeconomic status and fat percentage) have been taken into account in the statistical analysis*

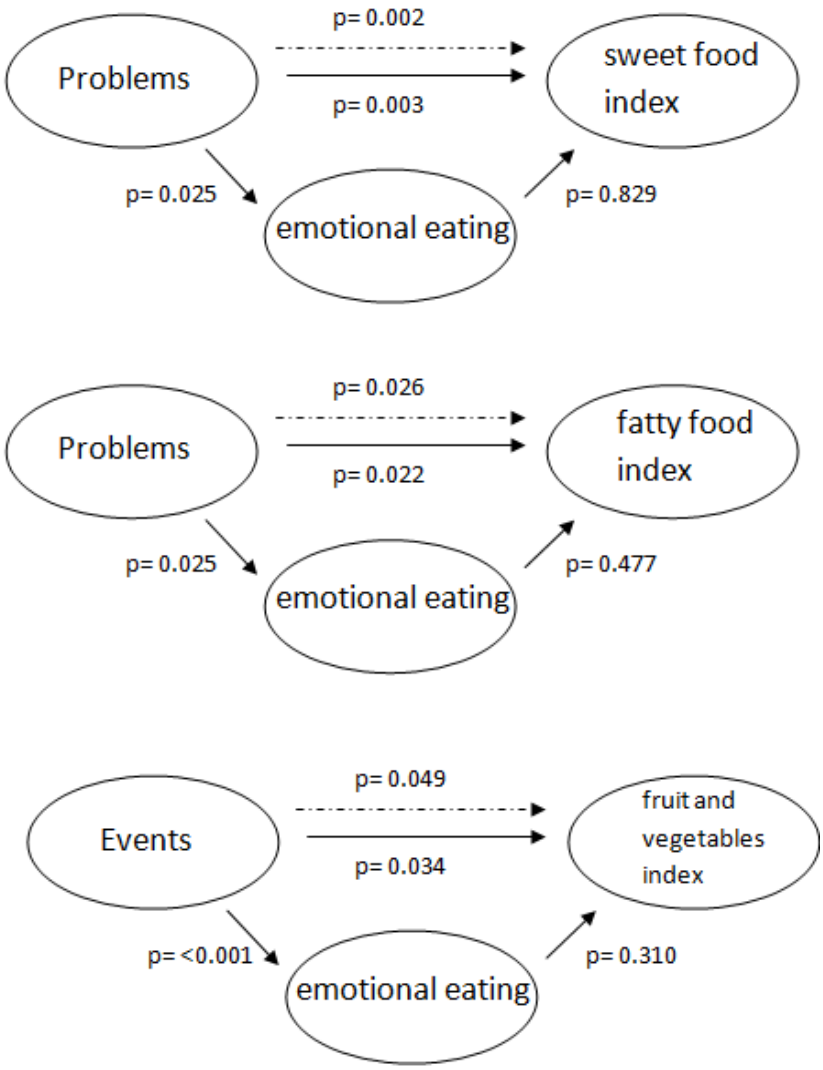


Figure 23: Mediation models (for which at least two out of four conditions were met): eating behaviour (emotional eating) as mediator in the stress – dietary patterns relation

The solid lines show the regression of the direct effect. The dashed lines show the regression corrected for emotional eating.

Discussion

This study has shown that stress in children (operationalized in three variables after PCA analyses on several stress-related questionnaires) was associated with emotional eating. Moreover, stress was also associated with an unhealthier dietary pattern (higher consumption frequency of fatty foods, sweet foods, and lower consumption frequency of fruit and vegetables). Nevertheless, emotional eating behaviour did not mediate the stress – dietary patterns relationship as emotional eating behaviour was not associated with the dietary patterns.

Stress and dietary patterns

Overall, stressed children had an unhealthier diet. The stress factor Problems showed the strongest association with the child's dietary patterns, especially when examining the sweet food index and the fat food index. Furthermore, children with a higher score on the second stress factor Events had a lower fruit and vegetables food index. The third stress factor Negative emotions was not related with any of the food indices.

In literature, this stress – dietary patterns relationship has been indicated before in adolescents: adolescent's perceived stress has been associated with more fatty foods, less fruit and vegetables and more snacks (Cartwright, Wardle et al. 2003) and lower overall diet quality (De Vriendt, Clays et al. 2011) and problem behaviour has also previously been associated with higher fatty food intake (Simon, Wardle et al. 2003). This supports the theory of stress induced eating of 'comfort foods' as either a stress coping strategy (escape) or because of the eating-induced stress reduction and associated reward feelings (Dallman, Pecoraro et al. 2003, Adam and Epel 2007). Nevertheless, this has seldom been studied in young children. To our knowledge, only one study in preadolescents demonstrated more snacking with more perceived stress (Jenkins, Rew et al. 2005) and one study showed more snacking in response to laboratory stressors (Roemmich, Wright et al. 2002). Our findings add to this knowledge on the relationship between stress and an unhealthier diet, thereby differentiating for more food indices and more stress variables.

In stress-induced changes of eating patterns, some variability across stress factors may exist. After all, the arousal (depression versus anger and fear), intensity (high versus low) and valence (positive versus negative) of emotions or stressors may

influence emotional eating behaviour differentially, complicating the stress - diet research (Macht 2008). Differences may also exist in diffuse versus clear-cut stress. In the case of diffuse or vague stress like daily events, people cannot allocate the stress or emotion and will be more prone to use eating as a way of coping. On the other hand, well-defined stress like strong emotions might not stimulate or even lower the motivation to eat (Macht 2008). Finally, apart from changes in dietary choice, also changes in overall food intake during stress may occur (Macht 2008), but this was not tested in our study.

Stress and emotional eating behaviour

Stress – emotional eating behaviour associations were positively significant for all three stress concepts. Indeed, children's and adolescents' negative emotions (depression and anxiety) and problems have been associated with emotional eating in literature (Braet and Van Strien 1997, Goossens, Braet et al. 2009, Nguyen-Rodriguez, McClain et al. 2010).

Apart from variability across stress factors, there is apparently also variability across individuals as they show different emotional eating behaviours (Macht 2008). The relationship that was found between stress and enhanced emotional eating is generally accepted, since emotional eating is defined as overeating in reaction to emotional arousal. People with an emotional eating attitude have learned to label the negative feelings of stress as 'hunger' (Bruch 1964). Furthermore, eating may be used as a way to cope with stress and it may also induce stress reduction and increase reward feelings (Dallman, Pecoraro et al. 2003, Adam and Epel 2007).

Emotional eating behaviour and dietary patterns

No significant associations were seen between emotional eating behaviour and dietary patterns. Nevertheless, some preliminary although contradicting evidence exists in literature. For preadolescent children, no studies with similar analyses were found, although two studies should be mentioned. First, in a study using another emotional eating behaviour questionnaire (i.e. the Emotion-Induced Eating Scale), emotional eating has been associated with more sweet foods, but not with fatty foods (Striegel-Moore, Morrison et al. 1999). Second, using the parental-report DEBQ, emotional

eating in children was related with a more fatty and sweet food consumption frequency (Braet and Van Strien 1997). In adolescents, some researchers also found more sweet food consumption in emotional eaters (Nguyen-Michel, Unger et al. 2007), while others not (Wardle, Marsland et al. 1992) or only in girls (Elfhag, Tholin et al. 2008). In a laboratory study, snacking was high in emotional/external eaters (Moens and Braet 2007). Indeed, emotional eating has been convincingly hypothesised to increase the intake of comfort foods (Macht 2008).

Several factors may explain the unexpected absence of an emotional eating behaviour – dietary patterns relationship. First, the diet of primary school children is still highly influenced and controlled by parents. Therefore, stressed children may have a higher desire to eat, but do eventually not get access to the food via their parents or at least not that their parents know. Indeed, studies have shown that children's diet and emotional eating behaviour is correlated with that of their parents (Longbottom, Wrieden et al. 2002, Brown and Ogden 2004, van Strien and Bazelier 2007). Nevertheless, a recent meta-analysis/review has shown that the parent-child relation should not be overstated and that there is a trend of diminishing influence the last decade by changing society (i.e. increasing importance of other influencing players like school and peers) (Wang, Beydoun et al. 2011). Moreover, the intake of sweet foods in 12-year olds was influenced by their emotional eating behaviour above the parental influence. In this context, age could be an important factor as in 7-9y old children the perceived parental eating restriction could prevent the child's emotional eating more than in the older group (van Strien and Bazelier 2007). The parental influence thus seems to be higher in young children.

Furthermore, emotional eating behaviour and dietary patterns have been reported by different reporters, respectively children and parents. Associations were highest when data from the same reporter was used: parental report of their child's problems and dietary patterns, children's report of their events and emotional eating behaviour.

Moreover, some limitations on questionnaires should be considered. The FFQ is not quantitative as portions were not assessed. As stress might sometimes change total food intake (Macht 2008), the real influence on the dietary patterns could be obscured by total intake. On the other hand, the DEBQ has also many questions on the intention to

eat (e.g. do you have more appetite in that situation) instead of the factual act of eating (=giving in to the desire). As non-overweight children might more closely control their desire (Moens and Braet 2007), the relationship between emotional eating behaviour and the actual diet could be absent in our low-overweight group. Using another questionnaire with focus on the act of eating instead of only the desire (the Emotion-Induced Eating Scale), emotional eating was indeed related with more sweet food intake (Striegel-Moore, Morrison et al. 1999). Finally, different clusters of emotional eating behaviour have previously been shown (e.g. dieters with emotional eating and those without) (Lindeman and Stark 2001). As different eating behaviours may result in different dietary patterns, clustering of eating behaviours could be a complicating factor.

Sex differences

We noted sex differences in 'stress' experiences. Although no gender differences were found for dietary patterns and emotional eating behaviour, sex differences were present in the relation of stress versus dietary patterns and emotional eating behaviour. More specifically, the three "stress – dietary patterns" relations were only significant for girls while the association between the stress – emotional eating were significant in both boys and girls but depending on the used stress factor.

Indeed, sex differences have been shown in children's psychological functioning and development (Crick and Zahn-Waxler 2003). Also, different comfort food preferences may exist across sex: women tend to prefer snack-related comfort foods while men preferred more nutritious meal-related foods (Wansink, Cheney et al. 2003). Research on those sex differences is still ongoing. Consistent with our results, stress and depression have been related to dietary patterns in female students but not in male students (Mikolajczyk, El Ansari et al. 2009).

Mediation

Only two out of four mediation conditions were met as the relation emotional eating behaviour – dietary patterns was absent. Therefore, emotional eating was no mediator in the stress – dietary patterns relationship. Research considering stress, emotional eating behaviour and dietary patterns together is scarce. To our knowledge, emotional eating behaviour has only been investigated as mediator in one study in 3714 adults. These

authors have shown emotional eating as mediator in the relation between depression and consumption frequency of sweet energy-dense food, but not in non-sweet energy-dense food and fruit/vegetables (Konttinen, Mannisto et al. 2010). In children, one relevant study examining our hypothesis was found, though a question on eating as a coping mechanism instead of an emotional eating behaviour questionnaire was used. In those children between 8 and 13 years old, mutual correlations between stress, unhealthy food and eating as coping were detected, but mediation was not examined (Jenkins, Rew et al. 2005). Another study in 9 to 12 year old children found relations of emotional eating behaviour with both diet and stress, but reported no information on stress versus diet (Braet and Van Strien 1997).

Strengths and limitations

As far as we know, this is the first study examining the mediation effect of children's emotional eating behaviour in the relation stress – dietary patterns. At this moment, only one study has already demonstrated this in adults (Konttinen, Mannisto et al. 2010). Major strengths are the direct measurement of dietary patterns, the multi-informant approach and the use of several aspects of dietary pattern and stress.

Still, some limitations have to be mentioned. Because of the quite large questionnaire battery, the responder burden was already high and no information on parental control and parental dietary habits was obtained. Secondly, the study population had a rather high socioeconomic status, leading to an overall healthy diet and low overweight percentage. Consequently, our results cannot be generalized to the overall population. Thirdly, dietary information was obtained by a FFQ, giving no qualitative information on total dietary intake. Nevertheless, using a FFQ has the advantage of showing the habitual diet as dietary recalls can be biased by exceptional days. Furthermore, as stated in the discussion, the DEBQ is not only focussing on the factual act of eating in the absence of hunger, but especially on intentions to eat in those situations. Finally, as the analyses were based on cross-sectional data, the mediation model is not a sound argument for causality. The "stress – dietary patterns" association could be bidirectional: diet and some nutrients in particular can also influence mood and stress reactivity (Gibson 2006).

Conclusions

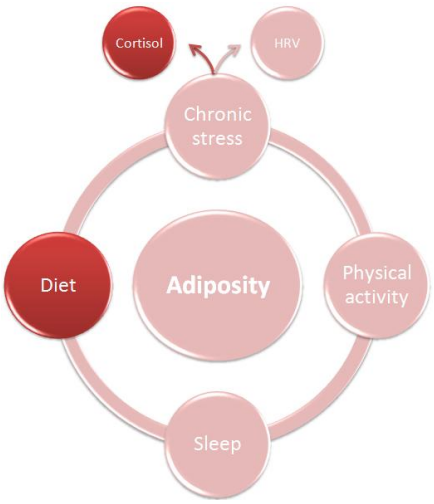
Stress was associated with emotional eating and an unhealthy dietary pattern and could thus be a trigger to overweight, also in children. We have shown that differences may exist between different constructs of stress (problems, emotions and events). Events were mainly associated with emotional eating. Problems were mainly associated with more frequent intake of fatty foods and especially sweet foods. On the whole, these results highlight the importance of stress reduction (e.g. by age-appropriate sleeping times and enough exercise) and the related unhealthy behaviour. On the individual level, parents and children should be made aware that stress can influence emotional eating behaviour so they can pay attention or anticipate to this behaviour. Children should be trained on their stress coping skills such as problem solving thinking or asking help instead of seeking solace in food. Nevertheless, as no association was found between emotional eating and dietary patterns, emotional eating behaviour was no mediator in the stress – dietary patterns association in children. Further studies should examine the underlying pathways in more detail.

3.

Relation between salivary cortisol as stress biomarker and dietary pattern in children

Michels N, Sioen I, Braet C, Huybrechts I, Vanaelst B, Wolters M, De Henauw S.

Psychoneuroendocrinology 2013; 38:1512-20.



Stressed is desserts

spelled backwards.

ABSTRACT

PURPOSE: Psychological stress has been suggested to result in hormonal effects (e.g. changes in cortisol pattern) that may change food selection in unhealthy ways. This study examines whether children's dietary pattern is indeed related to salivary cortisol levels.

METHODS: In 323 children (5-10 years old) participating in the Belgian ChiBS study, salivary cortisol samples, a biomarker for stress, was sampled when waking up, 30 and 60 minutes after wake up and in the evening on two consecutive weekdays. Data on the children's dietary pattern (frequency of sweet foods, fatty foods, snacks, fruit and vegetables) was collected with a food frequency questionnaire. Multilevel time modelling was used with adjustments for sex, age, body mass index, parental education and wake up time.

RESULTS: Higher overall cortisol levels and a large cortisol awakening response (CAR) were associated with more frequent consumption of sweet foods. A steeper diurnal cortisol decline was associated with a higher sweet, fatty and snack food consumption frequency. No associations with fruit and vegetables consumption were found.

CONCLUSIONS: High cortisol levels were linked to an unhealthier dietary pattern (more fatty food, snacks and especially sweet food). This supports the theory of cortisol-induced comfort food preference and strengthens the stress-diet relation.

Introduction

Psychological stress has been suggested to change food selection into unhealthy choices and potentially trigger overweight (Bjorntorp 2001, Dallman, Pecoraro et al. 2003, Torres and Nowson 2007). Indeed, stress has been associated with emotional eating behaviour, overall energy intake and an unhealthy dietary pattern (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Macht 2008). The theory of stress-induced craving for so-called 'comfort foods' (energy-dense foods high in sugar and fat content) explains stress-related eating as a way of avoidant stress coping, eating-induced stress reduction or because of the reward feelings associated with comfort foods that change food salience (Dallman, Pecoraro et al. 2003, Adam and Epel 2007).

One of the major neuroendocrine systems adapting the organism to stress situations is the hypothalamus-pituitary-adrenal (HPA) axis with cortisol production as hormonal end product. Cortisol secretion has a circadian rhythm with lowest levels in the first half of the night and a peak in the early morning. Apart from this circadian rhythm, a cortisol awakening response (CAR) is elicited by a quick cortisol increase within 30 minutes after wake up (Fries, Dettenborn et al. 2009). In general, the CAR is speculated to be an anticipation of the upcoming day by activation of memory representation and by orientation in time and space. Next to the single cortisol values, cortisol patterns such as this CAR and the diurnal slope (decline from morning to late evening) can serve as an index for adrenocortical activity on longer term.

The associations that have been reported between stress-exposure and cortisol levels are quite complex, giving conflicting hypotheses on the mechanism at work with both hyper- and hypo-cortisolism in response to stress. A hyper-/hypo-cortisolism hypothesis was published suggesting that recent exposure to a stressor may initially elevate cortisol levels (hypercortisolism with high morning cortisol and steep diurnal slope), while the HPA axis may develop a counter-regulatory response of cortisol lowering after extended stress exposure (Heim, Ehlert et al. 2000). A lower diurnal slope (mostly caused by low morning and/or high evening values) has been suggested as a less adaptive profile associated with stress, although with sometimes contradictory findings (Kristenson, Garvin et al. 2012). Furthermore, a recent meta-analysis found a global positive relation of the CAR with life stress (Chida and Steptoe 2009). Apart from the cognitive appraisal of upcoming events, the higher CAR may reflect a dysregulation of the cortisol axis resulting from previous stress exposure. In a previous

analysis based on self-reports, the stress-cortisol relations found in our child sample was more in line with the stress hypercortisolism hypothesis as self-reported stress was related to higher cortisol values and a steeper diurnal decline (Michels, Sioen et al. 2012).

Interestingly, this stress biomarker cortisol has been hypothesised as an appetite-stimulating hormone leading to stress-related dietary changes. The effect of cortisol on food consumption is mainly reward based: increased food salience and reward feeding using comfort foods. Cortisol can directly influence the reward pathways (e.g. in the mesolimbic system) through increased levels of opioids (role in hedonic evaluation of food) and dopamine (role in motivational aspects of eating). Furthermore, the cortisol effect may also be indirect through its influence on other hormones (e.g. insulin, leptin and NPY) that regulate appetite and reward (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Torres and Nowson 2007, Epel, Tomiyama et al. 2012).
As suggested by Torres and Nowson (2007), studying this interesting pathway is relevant and measuring a biological marker of stress (i.e. cortisol) will increase our understanding of the physiological mechanism underlying the stress-eating relation. The relation between cortisol levels and variables related to eating remains largely unexplored as subjective stress measurements have commonly been applied in stress research. Although stress questionnaires have been related to cortisol levels, variability in cortisol stress-response exists due to the nature of the stressor (social or physical) and to the person facing it (emotional response and psychiatric sequelae) (Miller, Chen et al. 2007). Consequently, measuring an objective marker of stress to examine this stress-diet relation is recommended.

Consequently, the present study aims to unravel the hypothesised detrimental effects of high cortisol levels (as a consequence of stress) on the dietary pattern in children. More specifically, three indices of unhealthy food consumption (sweet food, fatty food and unhealthy snacks, including both high fat and sugar) and one index of healthy food consumption (fruit and vegetables) were measured in a sample of preadolescent children. We hypothesised that high cortisol values and patterns (diurnal decline and CAR) might be associated with a higher unhealthy food consumption and a lower intake of fruit and vegetables. We focus in this study on children as the foundations of dietary habits are established from the ages of 3-4 years old (Singer, Moore et al. 1995) and may track into

adolescence and adulthood (Wang, Bentley et al. 2002, Mikkila, Rasanen et al. 2005). As such, this could elucidate opportunities for prevention.

Methods

Participants and general procedures

The subjects were 323 Belgian children (49% boys) between 5 and 10 years old participating in the baseline 2010 survey of the ChiBS study. Detailed aims, design, methods, population and participation characteristics can be found in chapter 2 "Methodology". As salivary cortisol sampling was an optional measurement module and some food questionnaires had missing data, only 323 of the 523 ChiBS children (=62%) were included for the current paper. The excluded sample did not differ in sex, age, socio-economic status and body composition.

Dietary patterns

Parents reported on their child's dietary pattern by completing a Food Frequency Questionnaire (FFQ). More information on the FFQ can be found in chapter 2 "Methodology". To identify dietary patterns, four indices on dietary pattern were computed by summing up the frequency of consumption of separate food items. A food index for (1) 'sweet foods' (i.e. sweet drinks, jam, honey, sweet breakfast cereals, sweet snacks), (2) 'fatty foods' (i.e. fried potatoes, chocolate- or nut-based spreads, high fat dairy, mayonnaise and mayonnaise-based products, cheese, fat meat preparations, butter, high fat snacks), (3) 'unhealthy snacks, including foods that are fat and/or sweet' (i.e. chocolate and chocolate bars, candy, biscuits, cake, ice-cream, chips) and (4) also a healthy food index for 'fruit and vegetables' (i.e. fruit, freshly squeezed fruit juice, vegetables) was calculated. To distinguish between fat, sweet and salty snacks, the snack index was further divided in fat & sweet snacks (chocolate and chocolate bars, biscuits, cake, ice cream), sweet/non-fat snacks (candy) and salty snacks (chips).

Salivary cortisol

Saliva was collected at home via Salivette synthetic swabs (Sarstedt, Germany) immediately after wake up (T0), 30 minutes after wake up (T30), 60 minutes after wake up (T60) and in the evening between 7 and 9 PM (Tev). More details on sampling and analyses can be found in chapter 2 "Methodology".

Possible confounders

Children's BMI and parental education were collected as reported in chapter 2 "Methodology". Children's sex and awakening time were reported by the parents. Children's age was calculated from birth date and examination date.

Statistical analyses

Due to a skewed distribution, cortisol concentrations were transformed by the natural logarithm.

Hierarchical linear modelling (HLM) was used to analyse the relation between the children's cortisol pattern and their dietary pattern, adjusted for potential confounders (sex, age, BMI, parental education and wake up time). HLM is a variant of multiple linear regression useful for data with a nested design, which is the case in this study as repeated cortisol measurements were obtained for each participating child. Therefore, a two-level model on the dependent variable 'cortisol' was used with the intra-individual parameters modelled at level 1 (cortisol pattern created by time of day) and the inter-individual parameters (questionnaire data and the personal characteristics as possible confounders) at level 2. The HLM was performed in the HLM/2L program (version 7.0), using an approach similar to the one published by Adam (Adam 2006). The significance level was set to $p<0.05$.

At level 1, the child's cortisol values were predicted by the time of day to estimate the shape of each child's cortisol curve during the day. Time of day values were expressed as 'number of hours since wake up' for each participant each day and centred to midday as six hours post-awakening. To characterise the CAR, a design variable was created assigning the value 1 to the sample taken 30 minutes after wake up, and the value 0 to all other samples. By representing the CAR as a separate variable in the model, the CAR became a separate coefficient that could be predicted independently of the other parameters, such as the diurnal slope. A dummy variable for day of measurement (first or second day) was included to account for possible systematic cortisol differences across days. The reported day-specific wake up time, considered as a cortisol-confounder, was also included as a level 1 parameter because of its day-dependence.

Questionnaire data on dietary pattern was entered as a level 2 predictor of each of the relevant level 1 predictors (intercept, slope and CAR) and these models were adjusted for potential confounders (sex, age, BMI, parental education and wake up time). Because of

multicollinearity, the dietary pattern variables were not entered simultaneously. Random error at level 2 was significant and as such included in the model.

To further assure whether the significances for diurnal cortisol slope were due to the pure decline parameter or rather due to changes of overall cortisol levels, non-hierarchical analyses were done on mean relative diurnal decline and the area-under-the-curve with respect to the ground (AUCg), summary variables that are often used in the literature. The relative diurnal decline was computed as "(awakening cortisol – evening cortisol) / awakening cortisol". The AUCg was calculated following the definition of Pruessner, Kirschbaum et al. (2003) and represents hypo- or hyper-cortisolism. These linear regression analyses were corrected for age, sex, BMI and parental education.

Results

In total, 323 children were included in the analyses. Descriptive data on children's personal characteristics, salivary cortisol and dietary pattern is given in Table 26. No sex differences were seen in the descriptive data (data not shown). A low percentage of overweight (7%) was present in our population. In 72.2% of the children, at least one of the parents had a tertiary or higher educational level.

Table 26: Descriptive data on children's personal characteristics, their salivary cortisol values and dietary pattern.

	Median	IQR
Personal characteristics		
Age (years)	8.41	7.49 - 9.36
Average wake up time (h; decimal)	6:57h	6:42 – 7:07h
Body mass index (z-score)	-0.28	-0.85 - 0.27
Salivary cortisol (nmol/L)		
Immediately after wake up	12.10	9.48 - 15.24
30 minutes after wake up	12.35	9.50 - 15.69
60 minutes after wake up	8.08	5.86 - 10.84
Evening	1.71	1.18 - 2.50
Children's dietary pattern (times/week)		
Sweet food frequency (12 items)	29	21 – 38
Fatty food frequency (14 items)	25	18 – 33
Unhealthy snack frequency (5 items)	9	6 – 12
Fruit and vegetables frequency (3 items)	14	11 – 19

IQR= Interquartile range

Table 27: Hierarchical linear model showing associations between children's cortisol and dietary pattern corrected for sex, age, BMI and parental education (n=323)

Fixed effect	Coefficient	SE	p	Interpretation (%/scale point) [a]	Maximal effect [b]
Cortisol intercept					
Sweet food frequency	*0.0034*	*0.0014*	*0.015*	*0.34*	*45.22%*
Fatty food frequency	0.0021	0.0014	0.143		
Snack frequency	0.0019	0.0023	0.410		
Fruit and vegetables	-0.0002	0.0028	0.956		
Diurnal cortisol slope [c]					
Sweet food frequency	*-0.0005*	*0.0001*	*<0.001*	*-0.05*	*6.65%*
Fatty food frequency	*-0.0004*	*0.0002*	*0.002*	*-0.04*	*5.40%*
Snack frequency	*-0.0009*	*0.0002*	*<0.001*	*-0.09*	*7.56%*
Sweet & fat	*-0.0015*	*0.0004*	*<0.001*	*-0.15*	*6.60%*
Sweet, not fat	*-0.0020*	*0.0006*	*<0.001*	*-0.20*	*4.20%*
Salty & fat	*-0.0035*	*0.0017*	*0.036*	*-0.35*	*4.90%*
Fruit and vegetables	0.0002	0.0002	0.706		
Cortisol awakening response (CAR)					
Sweet food frequency	*0.0038*	*0.0018*	*0.027*	*0.38*	*50.54%*
Fatty food frequency	0.0032	0.0022	0.143		
Snack frequency	0.0040	0.0039	0.303		
Fruit and vegetables	0.0047	0.0038	0.213		

Note. Natural logarithmic transformed cortisol values were used as dependent variable. Time since wake up (for diurnal cortisol slope), cortisol awakening response dummy (value 1 for sample 30 minutes after wake up), day of testing dummy (day 1 or 2) and wake up time were entered as level 1 variable. Dietary pattern (sweet, fat, snack and fruit and vegetables frequency) and day-independent confounders (sex, age, BMI z-score, parental education) were entered as level 2 predictor on each level 1 predictor. Variables in the final model do not represent the independent effect of each variable as all significant variables were entered separately.

[a] Since cortisol values were log-transformed, the following transformation has been applied to the B coefficient for interpretation: B%change=[exp(B)] - 1

*[b] Maximal effect in this population= % difference/scale point * range of this scale in the sample*

[c] Since the diurnal slope is negative, decreases in slope result in a steeper decline (=higher slope)

Table 27 provides the significant HLM results for the association between salivary cortisol and dietary pattern. Higher overall cortisol levels were associated with a higher frequency of sweet food consumption. A steeper diurnal cortisol decline was associated with a higher sweet food, fatty food and snack (all categories) consumption frequency. A larger CAR was associated with a higher sweet food consumption frequency. The same results were found when using a non-overlapping definition of sweet foods and fatty foods by only including sweet items that were not fat and fat items that were not sweet. No associations were found with the frequency of fruit and vegetables consumption and also no sex interaction effects (data not shown). Considering the maximal cortisol proportional differences between the lowest and highest consumption frequency in our population (=maximal effect), the cortisol-diet associations were most relevant for overall cortisol levels and the CAR.

To graphically represent these cortisol-food relations, estimated marginal means for salivary cortisol over the four time points are presented in Figure 24. Analyses were done using the logarithmic values, but the values were back-transformed for representation. Higher cortisol values, higher CAR and a steeper slope were clearly visible for the children with a high sweet index. Furthermore, a slightly steeper diurnal slope for a high sweet food index or fatty food index may be explained by the higher morning and slightly lower evening cortisol values.

Finally, linear regression analyses on the mean relative diurnal decline and the AUCg are represented in Table 28. These analyses confirmed the findings of Table 27: high sweet food and snack consumption were associated with a steeper diurnal decline and high sweet food consumption with an overall hypercortisolism (positive AUCg). In contrast to Table 27, fatty food consumption was not significantly associated with the decline, which might be due to a mix of a less strong fatty food-cortisol relation and the lower statistical power of linear regression in comparison to the HLM model.

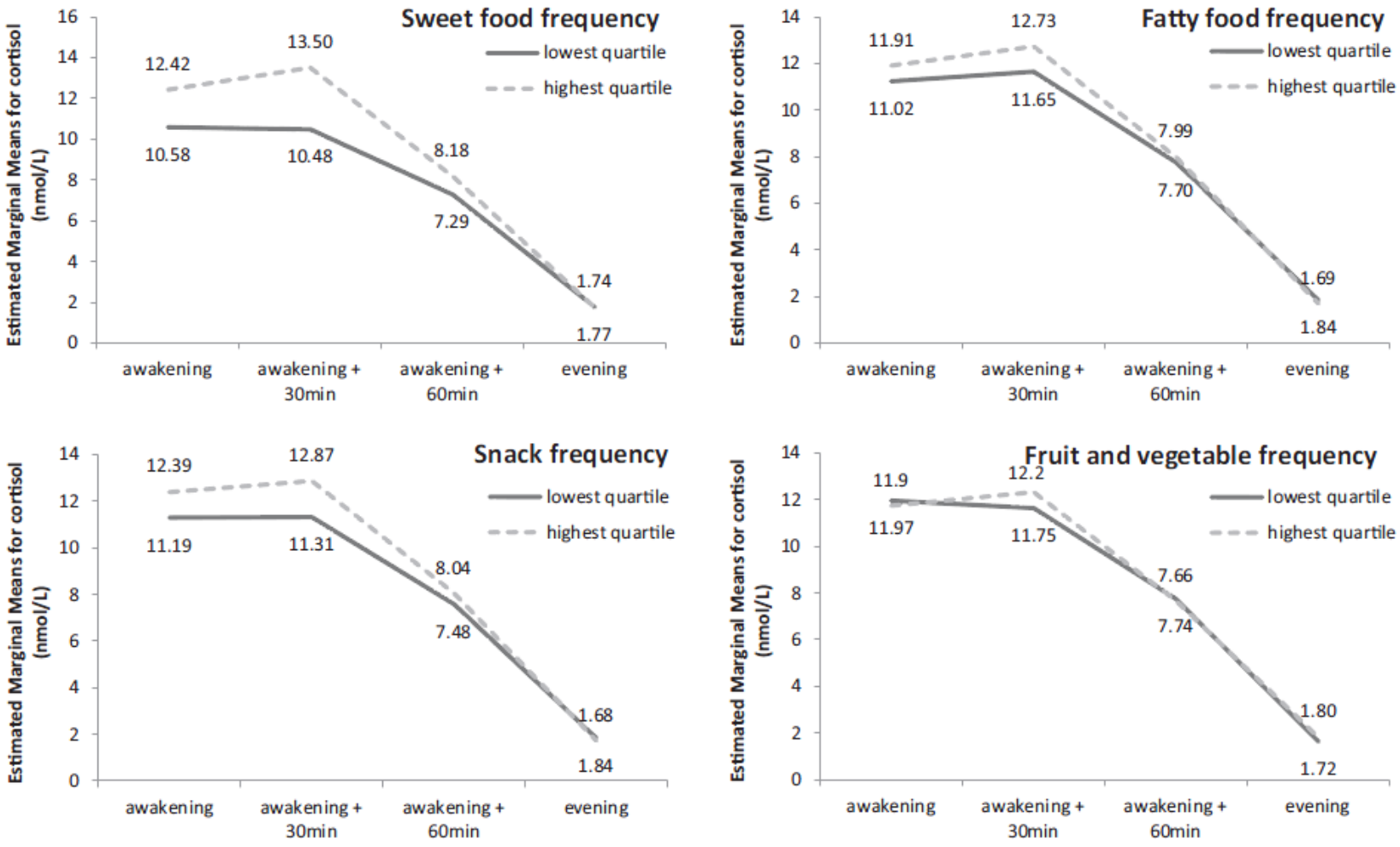


Figure 24: Estimated Marginal Means for salivary cortisol over the four time points depending on the food indices (lowest quartile versus highest quartile).

Table 28: Linear regressions showing associations between cortisol patterns (relative deline and AUC) and dietary pattern corrected for sex, age, BMI and parental education

	Relative decline		**Overall AUCg**	
	Bèta	***p***	**Bèta**	***p***
Sweet food frequency	*0.114*	*0.043*	*0.160*	*0.005*
Fatty food frequency	0.094	0.083	0.024	0.680
Snack frequency	*0.141*	*0.015*	0.043	0.458
Sweet & fat	*0.155*	*0.007*	-0.034	0.562
Sweet, not fat	*0.115*	*0.041*	0.076	0.165
Salty & fat	0.072	0.206	0.014	0.370
Fruit and vegetables	-0.071	0.218	-0.045	0.430

Relative decline= (morning cortisol – evening cortisol)/ morning cortisol; AUCg= area under the curve with respect to the ground representing overall cortisol

Discussion

This study explored whether cortisol could be one of the underlying factors in the effect of stress on diet. Interestingly, the salivary cortisol patterns (measured at home thus no laboratory stress exposure, only naturalistic daily stressors) of this population of children aged 5 to 10 years showed indeed a clear link with consumption frequency of selected food groups. Hypercortisolism shown by higher cortisol levels, steeper diurnal slope and more prominent CAR was associated with an unhealtier diet: a higher sweet food, fatty food and snack consumption frequency.

In the literature, self-reported stress has frequently been associated with an unhealthy dietary pattern. Although research in children and adolescents is less frequent, results have been replicated in these age groups. Adolescent's perceived stress has been associated with more fatty foods, more snacks and less fruit and vegetables consumption (Cartwright, Wardle et al. 2003) and lower overall diet quality (De Vriendt, Clays et al. 2011). Furthermore, problem

behaviour (= psychological problems) as measured with the Strength and Difficulties Questionnaire has been associated with more fatty food consumption (Simon, Wardle et al. 2003). Also in preadolescents, more snacking was demonstrated with more perceived stress (Jenkins, Rew et al. 2005) as well as in response to laboratory stressors (Roemmich, Wright et al. 2002). Although these studies confirm the stress-diet hypothesis, it is still not demonstrated that a biological mechanism underlies the assumed association.

Recent research already explored dietary effects of cortisol. Elevated cortisol levels after infusion of corticotrophin-releasing hormone stimulated food consumption in healthy adults (George, Khan et al. 2010). Also, so-called high "stress-reactors" (subjects with a higher cortisol increase after stressor) exhibited more overall stress-induced snacking in laboratory conditions (Epel, Lapidus et al. 2001) and when encountering daily hassles (Newman, O'Connor et al. 2007) compared to low stress-reactors. Remarkably, literature comparing basal cortisol levels with dietary patterns is very scarce. Urinary cortisol levels have been associated with higher fat intake in a Puerto Rican population (Laugero, Falcon et al. 2011). In another study, an association has been found between CAR and fat consumption in men, and protein consumption in women when they could choose from a buffet (Therrien, Drapeau et al. 2008). In conflict with our results, higher fat consumption in women has also been associated with a flat diurnal slope (morning sample was not at awakening) (Garcia-Prieto, Tebar et al. 2007).

Indeed, studies in adults have suggested a steeper slope as a more dynamic and probably healthy response (Kristenson, Garvin et al. 2012). In our previous analyses on this study sample however, the stress-cortisol relations were more in line with the stress hypercortisolism hypothesis: higher cortisol values and higher patterns such as a steeper decline (Michels, Sioen et al. 2012). This might indicate that hypocortisolism in children is less frequent (Bevans, Cerbone et al. 2008) as some time is needed before the hypercortisolism counter-regulatory response is induced. Overall, both hypo- and hypercortisolism have been demonstrated as deviations from the normal functioning and much of the variability is attributable to the stressor and person characteristics (Miller, Chen et al. 2007). In our study, a steeper diurnal slope was associated with more sweet, fat and snack consumption. Interpreting the cortisol patterns in Figure 24, this steeper diurnal slope was mainly caused by higher morning cortisol samples, although also slightly lower evening cortisol concentrations were shown. Higher cortisol values were significantly associated with

more sweet consumption. This association was not significant for fatty food and snack consumption, although a trend towards higher morning cortisol with higher fat/snack consumption was obvious in Figure 24.

Several hypotheses exist on this cortisol-diet relation (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Torres and Nowson 2007). Apart from an overall change in energy intake (in most cases an increase), a tendency for consuming more comfort foods has been shown. The consumption of comfort foods might be stimulated by reward centre activation through cortisol-induced opioid increases and more indirectly through cortisol-induced insulin, leptin and NPY increases. Furthermore, these released hormones might also stimulate overall appetite (Adam and Epel 2007). In this study, we found evidence for the theory of cortisol associations with eating of 'comfort foods'. Some discussion exists on whether comfort food must be sweet or fat or both e.g. only sweet & fat items were more consumed in high cortisol-reacting women (Epel, Lapidus et al. 2001). In our study, children with higher cortisol patterns consumed especially more sweet items, but also more fat/non-sweet items, fat & sweet snacks and fat & salty snacks were consumed. These results might suggest that comfort food mainly includes unhealthy sweet items (not the healthy sweet fruit) but also fatty items and perhaps salty items, probably depending on personal preferences. Nevertheless, findings on salty items in this study must be interpreted with caution, as these items were also high in fat. No salty/low-fat items were included in the list as they are not commonly consumed by the Belgian children.

For public health, dietary guidelines are formulated in the prevention of chronic diseases (obesity, cardiovascular diseases and diabetes) with a focus on low consumption of energy dense foods like those high in sugar and fat, but also on high consumption of fruit and vegetables (WHO 2003). As stress has previously also been related to lower consumption of fruit and vegetables (Kiviniemi, Orom et al. 2011), our analyses were repeated using the healthy food index on fruit and vegetables. Nevertheless, no associations were found with cortisol levels. Probably, the stress-induced behaviour on fruit and vegetables consumption might be not or less strongly influenced by hormonal changes as fruit and vegetables are only indirectly linked with reward feelings. Indeed, fruit and vegetable consumption was previously related with self-reported stress levels, but not with urinary cortisol levels (Laugero, Falcon et al. 2011).

Strengths and limitations

This is one of the first studies examining the relation between dietary pattern and stress by using a direct and objective, biological measure of stress (salivary cortisol) instead of using subjective stress questionnaires. A major strength is the repeated cortisol measure which made it possible to consider several alternative parameters across the day, in contrast to a single measure. Consequently, we used appropriate multilevel statistics which are increasingly used and have their advantages when analysing cortisol data: a high tolerance for within- and between-subject variation in sampling time, simultaneous modelling of multiple cortisol parameters is possible (elevation, diurnal slope and cortisol awakening response) and they have added statistical power because of the within-person repeated-measures design (Hruschka, Kohrt et al. 2005). Correction has been done for confounders especially parental education level which is strongly associated with the consumption of high-fat and high-sugar foods (Fernandez-Alvira, Mouratidou et al. 2012).

Our main hypothesis was that stress might influence dietary pattern by cortisol. Nevertheless, reverse causation could happen: calorically dense food influencing cortisol. Apart from literature on the cortisol effect of carbohydrate-to-fat ratio (McCargar, Clandinin et al. 1989, Stimson, Johnstone et al. 2007), high carbohydrate (London and Castonguay 2009, Martens, Rutters et al. 2010) and high fat (Tannenbaum, Brindley et al. 1997) intake can increase basal cortisol and the cortisol stress response. Several underlying mechanisms exist by which energy-rich comfort foods may influence cortisol levels: (1) the active cortisol availability by changes in the binding to transport proteins (corticosteroid-binding globulin) and (2) the cortisol production by changes in cortisol releasing hormone (adreno-corticotropic hormone), by changes in the converting enzymes (11β-hydroxysteroid dehydrogenase-1) or by changed levels of cortisol-decreasing opioids. Also, adiposity (as a result of the high energy food intake, but also by itself) is positively linked with cortisol, especially in abdominal adiposity (Vicennati and Pasquali 2000, Mussig, Remer et al. 2010). Perhaps, the cortisol-diet relation is bidirectional and forms a vicious circle: elevated cortisol may predispose individuals to consume calorically dense food and consequently to obtain a higher adiposity, while this type of diet may then further increase the cortisol levels, sustaining this behaviour. Moreover, third actors that are both related to cortisol and diet can confound, mediate or moderate the cortisol-diet association e.g. dietary restraint as enhancer of the reward based eating (Adam and Epel 2007), cortisol as enchancer of stressor (family conflict or more general hassles) induced snacking (Newman, O'Connor et al. 2007), or mental health status such as depression as

confounder of the cortisol-diet relation if it could influence diet also independently of cortisol through a general health-compromising attitude (Fulkerson, Sherwood et al. 2004). As the study design was cross-sectional in the sense that diet and cortisol were assessed at the same time, it forces us to temper our findings as modest reports on a significant association between cortisol and diet.

Although we had an elaborated measurement of dietary pattern focussing on several aspects (sweet foods, fatty foods, snacks, fruit and vegetables), no quantitative information on total dietary or nutrient intake or portion sizes was available since FFQ was used. Nevertheless, using a FFQ has the advantage of showing the habitual diet as dietary recalls can be biased by exceptional days. This instrument has good psychometric properties (Lanfer, Hebestreit et al. 2011). Since the children were too young to report dietary intake themselves, our instrument still has the restriction that only parental report is available. After all, parents do not always have the overall picture of their children's food intake e.g. for food intake at school: although they generally know what is on the menu (on the school's website), they can only guess whether their child has eaten all ingredients and whether an unhealthy snack was chosen. This could result in under- or over-reporting. Research in older children (10-12y) has shown quite low agreement rates between parental and child report for e.g. fruit and sweet intake, although this was still appropriate for correct ranking of intakes (Roumelioti and Leotsinidis 2009). Even more optimistic, fruit and vegetable intake reported in a parental FFQ showed correlation coefficients with serum vitamin levels that were similar as those observed in dietary validation studies among adults (Byers, Treiber et al. 1993).

A final limitation is that we only used a subjective measure of time compliance in the salivary cortisol sampling since objective compliance measurements were not feasible in this large population. Nevertheless, we stressed the importance of timing; moreover, the exclusion of self-reported non-compliers improves the accuracy (DeSantis, Adam et al. 2010).

Conclusion

Salivary cortisol patterns that might reflect higher stress levels (hypercortisolism reflected by both overall higher levels, steeper diurnal slope and more prominent CAR) were associated with an unhealthier dietary pattern: higher fatty food and snack consumption frequency, but especially higher sweet food consumption (not with the healthy sweet fruits). These results suggest the cortisol-stimulated association with preference for comfort foods, strengthening

the link between the HPA axis and diet that further might trigger chronic diseases like overweight, cardiovascular diseases and diabetes. This highlights the importance of targeting both stress reduction (e.g. by age-appropriate sleeping time, enough exercise and appropriate stress coping skills) and the related unhealthy dietary behaviour in preventions. Consequently, parents and children should be made aware that stress can influence their dietary pattern so they can pay attention or anticipate to this behaviour. Nevertheless, future research should also consider other appetite inducing hormones to further unravel the hormonal pathways underlying the stress-diet relation.

4.

Longitudinal associations between children's stress and lifestyle: the ChiBS study.

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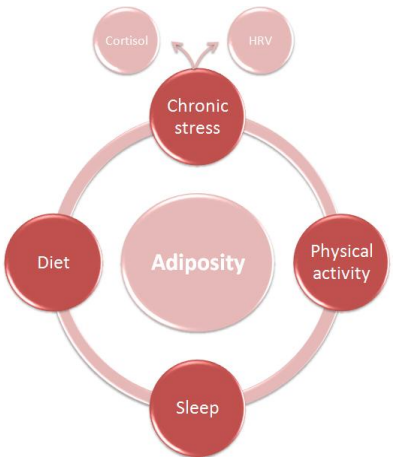
Health Psychology, in review.



Training gives us an outlet

for suppressed energy

created by stress

and thus tones the spirit.

ABSTRACT

OBJECTIVE: Psychosocial stress has been linked with an unhealthy lifestyle but the direction of this relation remains unclear. Does stress induces sleeping problems, comfort food consumption and a lower motivation for physical activity or do these unhealthy lifestyle factors enhance the stress level? This study will examine the bidirectional stress-lifestyle relation in children since the foundations of lifestyle habits start in childhood.

METHODS: In about 300 Belgian children (5-12y) participating in the ChiBS study, the relation between stress and lifestyle was examined over two years. Different stress related aspects were measured by questionnaires concerning negative life events, negative emotions and behavioural problems. Physical activity (by questionnaires and accelerometers), screen time, sleep duration, food consumption (sweet food, fatty food, snacks, fruit and vegetables) and eating behaviour (emotional, external, restrained eating) were assessed as lifestyle factor. Bidirectional relations were examined with cross-lagged analyses in Mplus software.

RESULTS: Unidirectionally, stress influenced lifestyle with increased physical activity, sweet food consumption, emotional eating, external eating and restrained eating. Sex and age were moderators in these stress-lifestyle relations. Moreover, one reversed direction effect (i.e. lifestyle influencing stress) was found with all three maladaptive eating behaviours increasing anxiety feelings.

CONCLUSION: Relations were mainly unidirectional: stress influenced children's daily lifestyle. Stress stimulates eating in the absence of hunger which could facilitate overweight. Consequently, families should realize that stress may influence children's diet, and problem-solving coping skills should be acquired. In contrast to recent findings, some stress aspects may also stimulate physical activity in the youngest children as a positive stress coping style.

Introduction

Mental health problems affect 10 to 20% of children and adolescents worldwide and are the leading cause of health-related disability in this age group with long-lasting effects throughout life (Kieling, Baker-Henningham et al. 2011). Mental health problems refer to a variety of manifestations including mood disorders, conduct problems and psychosomatic symptoms (WHO 1994). Besides social isolation (Mahon, Yarcheski et al. 2006) and school absence (Currie and Stabile 2007), one often neglected correlate of mental health problems is adopting an unhealthy lifestyle: changes in physical activity, sleep and diet. These lifestyle changes could lead to undesirable health consequences such as obesity, diabetes and cardiovascular risks. Moreover, these lifestyle factors have been shown to track into later life (Thorleifsdottir, Bjornsson et al. 2002, Mikkila, Rasanen et al. 2005, Ashcroft, Semmler et al. 2008, Telama 2009).

A model that is generally accepted in the origins of mental health is the vulnerability-stress perspective. This means that a central role is dedicated to psychosocial stress. Stress arises when the demands of a situation exceed an individual's ability to cope and resolve the problem, resulting in emotional, behavioural and cognitive disturbances (McCance, Forshee et al. 2006). Therefore, stress is for research purposes not only operationalized on the event level but also on the symptom level by measuring the daily problems and emotions.

Several lifestyle factors have been associated with psychosocial stress. These relations might be bidirectional: lifestyle can be influenced by stress and/or it can influence stress itself.

First, consistent evidence is found for an association between high stress and high sedentary screen time (Biddle and Asare 2011). However, these findings do not give clear indications on the causal mechanism. Remarkably, most research has focused on the opposite effect of physical activity on stress since activity can serve as a distraction from stress, it generates euphoric feelings, it increases social support and even decreases the stress response (Tsatsoulis and Fountoulakis 2006).

Second, sleep is more and more linked to stress. On neurological level, the regulation of sleep, behaviour and emotions are closely related: interactions between sleep, the amygdala and prefrontal cortex functions have been found that can result in deteriorated control of complex behaviour and emotions (Horne 1993, Gregory and Sadeh 2012). On psychological level, sleep is essential for proper emotion regulation while stress may result in problems to fall asleep due to rumination or next day anticipations. On a hormonal level, short sleep

increases stress hormones and these hormonal changes inhibit sleep, creating a vicious circle. A recent review showed that the sleep-stress relation in children is likely bidirectional but most evidence substantiated the effect of sleep on stress (Gregory and Sadeh 2012).

Finally, stressed people may eat increased amounts of unhealthy food since eating operates as a way to cope with stress (distraction) (Macht 2008). The assumed underlying pathway is the increased cortisol concentration that influences the reward and appetite pathways directly and indirectly (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Torres and Nowson 2007, Epel, Tomiyama et al. 2012). On the other hand, there is also evidence for the reverse pathway that food might enhance mood by opioidergic and dopaminergic neurotransmission (Gibson 2006) but this literature only covers adult populations. The association between stress and food consumption must be visible as well in people's reports on their eating behaviour. Schlundt demonstrated that different eating behaviours can be observed during a two-week period in people, such as external eating, restrained eating, emotional eating or binge eating (Schlundt, Taylor et al. 1991). In relation to stress, emotional eating is most important here. People with an emotional eating behaviour have learned to label the negative feelings of stress as 'hunger' (Bruch 1964) and will think about food as an escape from stress (Dallman, Pecoraro et al. 2003, Adam and Epel 2007). External eating refers to a personal trait reflected in a tendency to overeat in reaction to food cues whereas restrained eating means eating less than wanted when exposed to food (van Strien, Frijters et al. 1986). Both are also linked with stress since emotions have been hypothesized to impair cognitive control to external stimuli such as food (Macht 2008). However, the directionality remains unknown as this literature in children/adolescents is based on cross-sectional findings (Braet and Van Strien 1997, Goossens, Braet et al. 2009, Nguyen-Rodriguez, McClain et al. 2010, Hou, Xu et al. 2013). Guilt feelings might also cause enhanced stress as a consequence of these maladaptive eating behaviors (Bennett, Greene et al. 2013).

To conclude, evidence on the stress-lifestyle relation exists but the directionality is still unclear. Therefore, longitudinal studies are warranted. The present study aims to test the bidirectional longitudinal relation between stress and lifestyle (physical activity, sleep and diet) in children. After all, research on this topic is even more scarce in children. In doing this, the specificity will be tested by identifying which specific stress aspect (events, emotions or problems) predicts lifestyle. Multi-informant assessment of both the dependent and independent variables was chosen to avoid informant-based biases (van Dulmen and Egeland

2011). Moreover, moderating effects of sex and age are tested. After all, stress-related behaviours might not only differ depending on exposure characteristics (type and severity of stress/stressor), but also depending on individual characteristics (Becker, Berkley et al. 2008, Macht 2008, Roemmich, Lambiase et al. 2011, Balantekin and Roemmich 2012). Knowledge on the specificity and direction of the stress-lifestyle relation will help in the formulation of preventive strategies.

Methods

Participants and general procedures

Participants were Belgian children (50% boys) recruited for the longitudinal ChiBS study (2010-2012). The children were measured during 3 waves (i.e. measurement periods) with 1-year interval: in 2010, 2011 and 2012. At baseline, children were between 5 and 10 years old. More details on the ChiBS study can be found in chapter 2 "Methodology".

Overall, 523 children participated in February-May 2010 (T0), 455 in February-April 2011 (T1) and 330 in February-April 2012 (T2). We succeeded in including 326 children participated in all 3 waves and an extra 129 children in 2 of the 3 waves. Participants with and without complete data were compared using Little's Missing Completely At Random test (Little 1988). A non-significant χ^2 test statistic suggests that missing data are missing only in a random way and hence do not introduce any bias with regard to the central research question. Most measurements were performed in all three waves (2010, 2011 and 2012), but only two wave data is available for sleep (2010, 2012), accelerometry (2010, 2012) and eating behaviour (2011, 2012). The children that participated again in 2012 and those that dropped out between 2010 and 2012, did not differ on their stress score and lifestyle, except that those that dropped out had a higher screen time ($p=0.016$).

Stress questionnaires

Stress arises when the demands of a situation exceed an individual's ability to cope and resolve the problem, resulting in emotional and behavioural disturbances (McCance, Forshee et al. 2006). Negative events, negative emotions and behavioural problems were examined by questionnaires to cover the different aspects of stress. The three different stress aspects were studied separately. A total composite stress score (CSS) was then also calculated by summing

up the z-scores of the three stress aspects per child: one z-score for negative events, one z-score for negative emotions and one z-score for behavioural problems.

Negative events (child-reported at T0, T1 and T2)

The Coddington Life Events Scale for children (CLES-C) assesses the frequency and timing of 36 stressful life events relevant for this age group during the last year (see chapter 2 "Methodology"). For this paper, only the score of negative life events was used.

Negative emotions (child-reported at T0, T1 and T2)

Children were asked to report on their recent feelings of anger, anxiety and sadness as described in chapter 2 "Methodology".

Behavioural problems (parent-reported at T0, T1 and T2)

Parents were asked to complete the standardized 'Strengths and Difficulties Questionnaire' (SDQ) (Goodman 1997), reporting children's behavioural problems over the past 6 months. The statements were divided in subscales with higher scores reflecting difficulties or problems: peer problems, conduct problems and emotional problems.

Lifestyle factors

Physical activity and screen time (parent-reported at T0, T1 and T2)

Parents were asked about the usual physical activity and screen time of their child at each measurement wave. The sum of usual hours of physical activity outdoors and at sports club per week were used as a measure of physical activity. The reported usual number of screen time hours per week (e.g. television and computer time) was used as a measure of sedentary behaviour in the analyses.

Physical activity and sedentary behaviour (objectively measured at T1 and T3)

Physical activity was also measured with accelerometers for five consecutive days in 2010 and 2012. Sedentary and moderate-to-vigorous physical activity were expressed using percentages to correction for wearing time.

Sleep (parent-reported at T1 and T3)

Parents also reported in 2010 and 2012 the usual time of the child going to bed in the evening and getting up in the morning on weekdays and weekend days, from which the child's sleep duration was calculated.

Food Frequency Questionnaire (FFQ) (parent-reported at T0, T1 and T2)

Parents reported on their child's dietary pattern during the last 4 weeks by completing a Food Frequency Questionnaire (FFQ). More information on the FFQ can be found in chapter 2 "Methodology". To identify dietary patterns, four indices on dietary pattern were computed by summing up the frequency of consumption of separate food items. A food index for (1) 'sweet foods' (i.e. sweet drinks, jam, honey, sweet breakfast cereals, sweet snacks), (2) 'fatty foods' (i.e. fried potatoes, chocolate- or nut-based spreads, high fat dairy, mayonnaise and mayonnaise-based products, cheese, fat meat preparations, butter, high fat snacks), (3) 'unhealthy snacks, including foods that are fat and/or sweet' (i.e. chocolate and chocolate bars, candy, biscuits, cake, ice-cream, chips) and (4) a healthy food index for 'fruit and vegetables' (i.e. fruit, freshly squeezed fruit juice, vegetables) was calculated.

Dutch Eating Behaviour Questionnaire (DEBQ) (child-reported at T1 and T2)

Children had to fill in the 33-item DEBQ on their usual eating behaviour. Three types of eating behaviour can be identified in children: eating in response to negative emotions (emotional eating), eating in response to the sight or smell of food (external eating) and eating less than desired to lose or maintain body weight (restrained eating). In all three types of eating behaviour, the appropriate self-regulating mechanism of food intake is diminished or lost.

Possible confounding factors

Sex, age, socio-economic status and body mass index (BMI) were considered as potential confounding factors. Since correction for BMI did no modify the results, the results are shown without correction for BMI. After all, only 7% overweight was seen at baseline.

The children's sex and birth date were reported by the parent. BMI and parental education (to represent socio-economic status) were assessed as reported in chapter 2 "Methodology".

Statistics

Descriptive statistics Paired sample t-tests were used to analyse the evolution of stress and lifestyle factors over the three measurement times. A significance level of $p<0.05$ was used.

SEM Structural equation modelling (SEM) in Mplus (Muthén and Muthén 2007) with maximum-likelihood estimation was used to determine longitudinal associations between stress (the CSS, negative events, negative emotions and total problems) and lifestyle (measured and reported physical activity, measured sedentary activity, screen time, sleep duration, four dietary intake factors and three eating behaviours). A number of fit indices were used to evaluate the model: χ^2 test, the comparative fit index (CFI) and the root mean square error of approximation (RMSEA). χ^2 /df ratio of 2 or below, CFI values of 0.90 or above and RMSEA values of 0.06 or below were used as indicators of acceptable fit (Kline 2005).

Measurement model To correct for measurement error, three parcels were created in modelling total problems (using the subscales peer problems, conduct problems, emotional problems), negative emotions (using the subscales anger, anxiety, sadness) and the three eating behaviour variables (each parcels consisting of approx. 4 of the original questions of the specific eating behaviour). Other variables could not be modelled by parcels, since only manifest variables were available. For all models, indicators had significant and moderate to strong loadings on the latent factors, ranging from 0.44 to 0.91 (all $p<0.001$). Measurement models demonstrated a good fit to the data.

Structural model Cross-lagged models were used as shown in Figure 25, including 1) cross-lagged paths (e.g., from stress at baseline to lifestyle at follow-up) 2) autoregressive paths (e.g., stress at time 1 to stress at time 2) 3) correlations within waves. To control for possible confounding effects (age, sex and socio-economic status), paths were allowed from each of these three variables to all the constructs included in the structural models. The results of the cross-lagged associations between the 2011 and 2012 wave will mainly be interpreted since these are corrected for all the earlier factors and associations (Burkholder and Harlow 2003).

Multi-group analyses Multi-group comparisons were tested to investigate potential structural differences for the cross-lagged model depending on sex and age. Age was transformed in a categorical variable by creating two groups based on a median (one group <8, one group ≥8). The analysis was done by comparing the fit between the constrained model, in which the

structural relations between both groups were not allowed to vary and the unconstrained model, in which the structural relations were set free. The difference in chi-square statistic and CFI statistic between both models was calculated as follows: "constrained model statistic – unconstrained model statistic". Non-equivalence between boys and girls was considered in the case of a significant χ^2 difference and a CFI difference higher than 0.01.

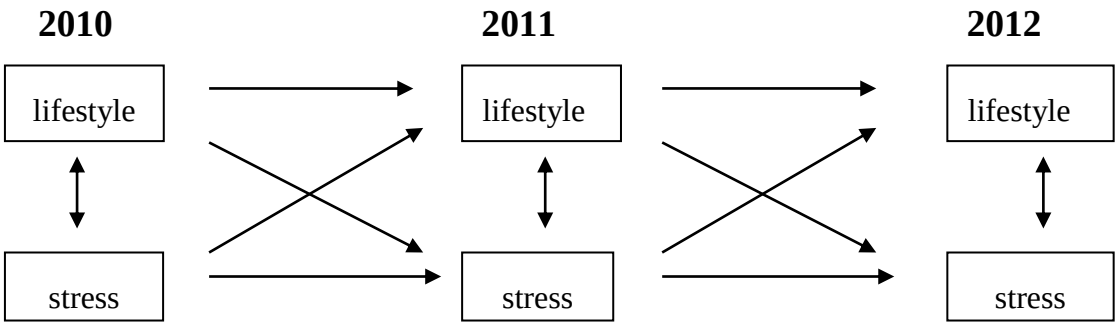


Figure 25: Model for the longitudinal cross-lagged models analysing the stress-lifestyle relation.

Results

Descriptive data

Descriptive data of the used stress and lifestyle parameters are shown in Table 29 (both median level and scores of participants who were in the 25^{th} or 75^{th} percentile). The underlying subscores for the stress aspects 'negative emotions' and 'total problems' are only shown for descriptive purpose. A significant increase in negative events and conduct problems, and a decrease in negative emotions was seen between 2010 and 2012. Changes were also seen in lifestyle, reflecting the natural evolution with age: increased screen time, sedentary time and restrained eating but a decreased reported physical activity, sleep duration, emotional eating and external eating. No changes in food consumption frequency were seen over time.

Table 29: Descriptive data of stress and lifestyle parameters at baseline and second follow-up

	2010			2012			p-value*	N
	P25	P50	P75	P25	P50	P75		
STRESS PARAMETERS								
Negative events (score)	10	40	73	24	63	106	<0.001	264
Negative emotions (0-30)	3	7	11	2	5	9	<0.001	265
anger (0-10)	1	3	5	1	2	3	0.006	265
anxiety (0-10)	0	1	3	0	0	2	0.004	265
sadness (0-10)	0	2	4	0	1	4	<0.001	265
Total problems (from SDQ) (0-30)	2	4	7	3	4	7	0.290	315
conduct problems (0-10)	0	1	2	0	2	3	0.002	315
peer problems (0-10)	0	1	2	0	1	2	0.062	316
emotional problems (0-10)	1	2	3	1	2	3	0.684	316
LIFESTYLE PARAMETERS								
Physical activity/inactivity								
Screen time (h/week)	7.3	11	15.3	8.5	12.3	17.8	<0.001	309
Reported activity (h/week)	9.8	14	19.5	9.5	13.5	17.5	0.057	298
Sedentary time by accelerometer (%)	47.3	51.9	56.1	53.1	58.8	63.5	<0.001	153
Moderate-to-vigorous activity time by accelerometer (%)	4.4	5.7	7.5	5.3	7.1	9.3	<0.001	153
Sleep								
Sleep duration (hours/night)	10.5	11	11.3	10.3	10.6	11	<0.001	258
Food consumption frequency (consumptions/week)								
Snacks	6	8	12	6	9	14	0.683	277
Fatty foods	19	25	34	20	27	37	0.576	258
Sweet foods	21	29	39	22	29	39	0.828	255
Fruit and vegetables	11	14	19	11	14	21	0.291	267
Eating behaviour (Dutch eating behaviour questionnaire)								
Emotional eating (1-5) [#]	1.4	1.9	2.5	1.2	1.6	2.2	<0.001	312
External eating (1-5) [#]	2.7	3.1	3.6	1.4	2.0	2.6	<0.001	312
Restrained eating (1-5) [#]	1.6	2.2	2.7	2.5	3.0	3.5	0.002	312

*[#] these parameters were not collected in 2010, data of 2011 are shown instead; * paired t-test p-value for individual change over 2010-2012; P25/50/75: 25th, 50th and 75th percentile; SDQ= Strengths and difficulties questionnaire*

Cross-sectional associations of stress and lifestyle

Although the main focus of this article was on the cross-lagged longitudinal associations, cross-sectional paths in this model also showed significances. Positive cross-sectional relations with stress were seen for screen time (with CSS and total problems), sweet intake (with CSS and total problems), fat intake (with CSS and total problems), emotional eating (with CSS and negative events, negative emotions and total problems), external eating (with CSS and negative emotions and total problems) and restrained eating (with CSS and negative events, negative emotions and total problems). Negative cross-sectional relations with stress were seen for objectively measured physical activity (with CSS and negative emotions) and for sleep duration (with CSS and negative emotions). No cross-sectional relations were found for consumption frequency of snacks and fruit and vegetables, sedentary time and the physical activity questionnaire.

Longitudinal associations of stress and lifestyle

Table 30 shows the significant longitudinal analyses for the effects of stress (CSS and three separate stress aspects) on lifestyle. Physical activity questionnaire data and external eating showed positive longitudinal associations with CSS. Physical activity questionnaire data, accelerometer data and external eating showed positive longitudinal associations with negative events. Physical activity questionnaire data also showed positive longitudinal associations with negative emotions.

Also some evidence for reversed direction of effect was found (i.e. effects of lifestyle on stress). No lifestyle effects on CSS were found but maladaptive eating behaviors (emotional, external and restrained eating) could increase anxiety feelings. This effect of emotional and external eating was significant in the total population (β=0.159, p=0.005 and β=0.158, p=0.032, respectively), while the effect of restrained eating was only seen in girls (β=0.256, p=0.014).

Table 30: Significant longitudinal effects of children's stress (in 2011) on lifestyle (in 2012)

		Fit indices	
	Bèta	CFI	RMSEA
Physical activity by questionnaire			
Composite stress score CSS	0.12*	0.912	0.034
Negative events	0.14*	0.909	0.044
Negative emotions	0.17**	0.915	0.045
Moderate-to-vigorous physical activity			
Negative events	0.15*	0.900	0.049
External eating			
Composite stress score CSS	0.13*	0.985	0.024
Negative events	0.17**	0.977	0.028

*The cross-lagged models are adjusted for age, sex, body mass index and socio-economic status.*P<0.05, **P<0.01, ***P<0.001; CFI= comparative fit index; RMSEA= root mean square error of approximation.*

Moderation in the longitudinal associations

Multi-group analyses in the cross-lagged models showed that age and sex were moderators in the effects of stress on lifestyle.

The sex differences are shown in Table 31. Restrained eating could longitudinally be predicted by total problems only in boys. Emotional eating could longitudinally be predicted by negative emotions only in girls. External eating could longitudinally be predicted by negative events only in girls.

The age differences are shown in Table 32. Stress (CSS and total problems) had a stimulating effect on sweet food consumption and restrained eating only in older children. The stimulating effects of stress (CSS and negative emotions) on physical activity were only seen in young children, while negative emotions even decreased physical activity in older children.

Table 31: Sex differences in longitudinal effects of children's stress aspects (in 2011) on lifestyle (in 2012)

	Boys		Girls	
	Bèta coefficient	p	Bèta coefficient	p
Emotional eating				
Negative emotions	-0.074	0.310	0.193*	0.022
External eating				
Negative events	0.024	0.809	0.319***	<0.001
Restrained eating				
Total problems	0.170*	0.022	0.016	0.846

*The cross-lagged models are adjusted for age, body mass index and socio-economic status.*P<0.05, **P<0.01, ***P<0.001*

Table 32: Age differences in longitudinal effects of children's stress aspects (in 2011) on lifestyle (in 2012)

	Young (6-8y in 2011)		Old (9-11y in 2011)	
	Bèta coefficient	p	Bèta coefficient	p
Physical activity by questionnaire				
Negative emotions	0.274	0.003**	0.054	0.453
Moderate-to-vigorous physical activity				
Composite stress score CSS	0.191	0.034*	0.045	0.586
Negative emotions	0.270	0.003**	-0.135	0.039*
Sweet food consumption frequency				
Composite stress score CSS	-0.007	0.932	0.192	0.011*
Restrained eating				
Total problems	0.012	0.486	0.041	<0.001***

*The cross-lagged models are adjusted for sex, body mass index and socio-economic status.*P<0.05, **P<0.01, ***P<0.001*

Discussion

This study in about 300 Belgian children confirmed the negative influence of stress on diet (sweet food consumption) and eating behaviour. In contrast, stress also had a positive longitudinal influence on physical activity. Sex differences were seen in the influence of stress on eating behaviour, while age differences were seen in the influence of stress on activity, diet and eating behaviour. Only minor evidence was found for reversed direction effects e.g. an effect of lifestyle on stress: only maladaptive eating behaviours influenced the emotional status (anxiety) of these children.

Apart from the differential impact of different stress aspects, also a composite stress score was examined. This CSS was associated with physical activity, sweet food consumption and external eating. Although the three aspects of stress (negative events, negative emotions and total problems) were collected over other timeframes (last year, generally, last 6 months) and by different informants, they were all associated with some lifestyle outcomes: negative events (child report) influenced activity and external eating; negative emotions (child report) influenced activity and emotional eating; and problems (parent report) influenced restrained eating.

When examining lifestyle in children, the parental control over children's lifestyle needs to be considered. This may explain the higher impact of stress on lifestyle factors with less parental control, such as psychological eating behaviour and physical activity. The impact was smaller or non-existing for lifestyle factors that are mainly controlled by the parents such as the factual dietary intake (parents influence the availability of food at home), sleep duration and screen time.

Physical activity

During stress, energy is mobilized as preparation for physical activity in the fight/flight reaction. Consequently, physical activity is the ideal stress reaction since it will use the mobilized energy and prevent energy storage (Tsatsoulis and Fountoulakis 2006). In contrast, stress might decrease physical activity due to a lack of time and motivation. Although most studies were cross-sectional, a longitudinal study in adolescents found a negative bidirectional relation between depression and physical activity (Stavrakakis, de Jonge et al. 2012).

Nevertheless, the composite stress score and more specifically negative emotions and events increased physical activity in our childhood sample. Of course, physical activity can be used as way to cope with stress since walking/running was reported by a group of children as a frequently used and efficient coping strategy (Chen and Kennedy 2005). Also in a laboratory experiment, physical activity increased but only in those that have a high usual level of physical activity (Balantekin and Roemmich 2012). When stratifying our analyses by age, the positive relation was only significant in the young group and even lower physical activity was found in the older group when confronted with negative emotions. This suggests that stress coping strategies are age-dependent. Perhaps, this positive finding will disappear when kids grow up into adolescents that have more time constraints for doing physical activity due to other priorities like schoolwork.

Not only the simple bidirectional relation between stress and activity has been studied, evidence exists that physical activity can serve as a buffer for stress effects on body composition (Yin, Davis et al. 2005), the metabolic syndrome (Holmes, Eisenmann et al. 2008) or even overall health (Gerber and Puhse 2009). This is an important health message since spending time for exercising might be considered as a waste of time when people are stressed although it seems the ideal way to relieve your stress.

Screen time

Apart from physical activity, also sedentary behaviour such as screen time might be influenced by stress. A recent review in children/adolescents reported an association between high sedentary screen time and poorer mental health (Biddle and Asare 2011). Literature also indicates that the stress influence on screen time could depend on usual screen time (Balantekin and Roemmich 2012) and on stress reactivity (Roemmich, Gurgol et al. 2003). Although we only found a cross-sectional relation between stress and higher screen time, interventions on screen time might be beneficial in the long term for other lifestyle factors. An experimental increased screen time in 8-12y old children resulted in a decreased physical activity and increased energy intake (Epstein, Paluch et al. 2002). Also, a recent review showed increased food intake in the absence of hunger while performing sedentary activities like screen time (Chaput, Klingenberg et al. 2011).

Sleep

Sleep is the metabolic antagonist of stress given its opposite effects on heart rate, blood flow and hormones. Indeed, a recent review concluded that associations between sleep and stress in children and adolescents are likely bidirectional, with sleep problems or insufficient sleep exacerbating emotional and behavioural problems, while mood disturbances and anxiety compromised sleep patterns (Gregory and Sadeh 2012). In our study, short sleep duration was only cross-sectionally related with negative emotions. Unfortunately, no longitudinal information on sleep quality (sleep latency, awakenings, night terrors) was collected. After all, sleep quality is less parentally controlled than sleep duration and most problem behaviours were related to sleep quality in a childhood population (Smedje, Broman et al. 2001). Moreover, it is suggested that the effects on sleep could be larger in adolescents (Gregory and Sadeh 2012).

Diet

In literature, perceived stress has been associated with more snacking in preadolescents (Jenkins, Rew et al. 2005) and with less fruit/vegetables and more snacks (Cartwright, Wardle et al. 2003) or an overall lower diet quality (De Vriendt, Clays et al. 2011) in adolescents. Also in response to laboratory stressors, preadolescents showed more snacking but especially those that were high in restraint, even when other stress coping behaviours were freely available (Roemmich, Wright et al. 2002, Balantekin and Roemmich 2012).

As hypothesized, stressed children had a less healthy diet, but this was only reflected in sweet food consumption, not in fatty food, overall snack food or fruit and vegetable consumption frequency. In a cross-sectional analysis in our population, sweet foods showed the best association with the stress hormone cortisol and stress questionnaires (Michels, Sioen et al. 2012, Michels, Sioen et al. 2013). Consequently, mainly the sweet taste might be defined as 'comfort food' in our paediatric population or alternatively, children might have more independence in the consumption frequency of sweet items.

The effects of stress on diet can be quite complex since variability exists across stress factors. Eating would mainly be stimulated by moderate emotions, while decreased intakes have been reported in very intense and high-arousal emotions (Macht 2008). Although these negative emotions influence eating behaviour (the intention to eat), they do not necessarily influence the factual act of eating (giving in to the desire). The higher independence older children have over their own food consumption compared to the higher parental control in younger children was reflected in stress-food relations being only significant in the oldest group.

Eating behaviour

Apart from food consumption, also eating behaviour was studied. Literature states that people can change their diet as a reaction on stress by maladaptive eating behaviour (Macht 2008). In literature, children's and adolescents' negative emotions and problems have been associated mainly with emotional eating (Braet and Van Strien 1997, Goossens, Braet et al. 2009, Nguyen-Rodriguez, McClain et al. 2010). Also increased external eating (Braet and Van Strien 1997, Hou, Xu et al. 2013) has been associated with stress and to a lower extent also more restrained eating (Hou, Xu et al. 2013).

In our study, relations were indeed seen with higher emotional, external and restrained eating. This depended on the used stress concept: emotional eating was increased by emotions, external eating was increased by events and restrained eating was increased by problem behaviour. In addition, a gender effect was seen: significant relations for emotional and external eating only in girls and for restrained eating only in boys. Probably, cultural values regarding boys and girls can facilitate or inhibit psychological eating. These eating behaviours finally have their effect on dietary intake. Moreover, these maladaptive eating behaviours increased anxiety feelings. Since this was the only observed effect of lifestyle on stress, a bidirectional relation was solely found for emotional eating. Nevertheless, this bidirectional relation might possibly lead to a vicious circle between stress and diet.

Strengths and limitations

A major strength of this study is the longitudinal design with cross-lagged analyses. In contrast to the literature that is mainly based on cross-sectional findings, it reveals the directionality of the relation which is important as the stress-lifestyle relation might be bidirectional. A second strength is the multiplicity of lifestyle factors (diet, eating behaviour, physical activity, sedentary time and sleep duration), measurement methods (both objective and subjective activity measures) and stress concepts (events, emotions, problems) that have been used by a multi-informant assessment. A third strength is the tested moderation by sex and age in the stress-lifestyle relation.

Despite the large battery of collected data, no longitudinal information on other possible predictors such as total energy intake (Macht 2008) and sleep quality (Kim and Dimsdale 2007) was available. Age and sex were tested as moderators but the unmeasured stress reactivity could also be an important moderator (Roemmich, Gurgol et al. 2003, Balantekin and Roemmich 2012). Moreover, a recent study found that stress was related to high variability in sleep duration, and not in mean sleep duration as was used in our study (Mezick, Matthews et al. 2009). Finally, our results in Belgian children cannot be generalized to the overall population since cultural variations have been described in stressors and coping (Chen and Kennedy 2005).

Conclusion

This study has shown that stress can influence children's lifestyle by especially deteriorating eating behaviour. In contrast to recent findings, some stress aspects can also stimulate contra-obesity behaviour, more specific by increasing physical activity. The observed relations were mainly unidirectional with only very limited evidence for effects of lifestyle on stress. The lifestyle behaviours that were influenced by stress depended on individual (sex and age) and exposure (type of stress concept) characteristics. As children grow older, they have more independence over food choices and more time constraints for physical activity. To prevent overweight, it therefore becomes increasingly important to make the environment (e.g. at home, at school etc.) an 'activity encouraging, healthy food zone' that minimizes opportunities for stress-induced eating. Children and their parents should be made aware that stress can influence their diet, and problem-solving coping skills should be highlighted. Although not all examined lifestyle factors were influenced by stress in our study, intervention programs influencing one factor may influence the others.

V.

ADIPOSITY RESULTS

1.

The association between childhood stress and body composition, and the role of stress-related lifestyle factors – cross-sectional findings from the baseline ChiBS survey

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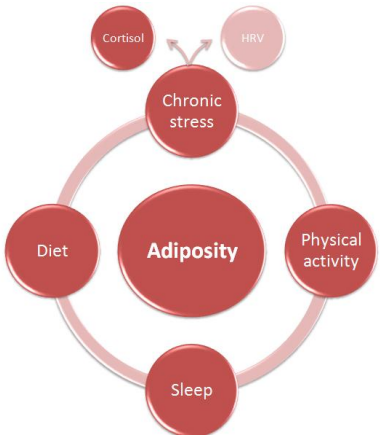


If stress burned calories,

I would be a supermodel.

ABSTRACT

OBJECTIVE: Stress has been hypothesised to be involved in obesity development, also in children. More research is needed into the role of lifestyle factors in this association.

METHODS: A total of 355 Belgian children (5-10 years old) participating in the baseline 'Children's Body composition and Stress' survey were included in this study. The following variables were studied: psychosocial stress (i.e. stressful events, emotions and behavioural/emotional problems, salivary cortisol), stress-related lifestyle factors (sleep duration; high-caloric snack, sweet food, fatty food and fruit & vegetables consumption frequency; physical activity and screen exposure time) and body composition parameters (BMI z-score, fat%, waist-to-height ratio (WHtR)). Using linear regression analyses (adjusted for sex, age and socio-economic status), the relation between stress and body composition and, more importantly, the possible moderating or mediating role of lifestyle factors was tested.

RESULTS: No association was observed between body composition and negative emotions, conduct and emotional problems and salivary cortisol. However, negative life events were positively and happiness was negatively associated with BMI z-score and WHtR. Peer problems were positively associated with WHtR and fat% in girls only. These associations were not significantly reduced after correction for lifestyle factors. Nevertheless, most lifestyle parameters (consumption of sweet food and snacks, screen time and sleep) moderated one or more stress – body composition associations, resulting in more significant relations in subgroup analysis.

CONCLUSION: Childhood stress was positively related to both overall and central adiposity measures with lifestyle factors acting as moderators but not as mediators. Thus, lifestyle could be a vulnerability factor in stress-induced adiposity, creating a perspective for multi-factorial obesity prevention, targeting stress and lifestyle factors in parallel.

Introduction

The prevalence of childhood obesity significantly increased during the last decades with currently around 110 million overweight and obese children worldwide (Moreno, Pigeot et al. 2011). Excessive caloric intake, insufficient physical activity and sleep deprivation are major lifestyle factors involved in the development of childhood obesity (Moreno, Pigeot et al. 2011). Moreover, a wide array of other – genetic and environmental – factors have been shown to be involved in the development of obesity; a recently identified potential predictor of overweight is chronic stress in children (Gundersen, Mahatmya et al. 2011, Pervanidou and Chrousos 2011).

Although children are not always recognized as being susceptible to stress, chronic exposure to stressful situations in children's school, family or interpersonal environment (further defined as 'psychosocial stress') is not uncommon and may adversely affect their physiological and psychological health (Teicher, Andersen et al. 2002, Schneiderman, Ironson et al. 2005, Cohen, Janicki-Deverts et al. 2007). Of special concern is the combined increase in the prevalence of childhood stress with the prevalence of psychosomatic complaints (Vanaelst, De Vriendt et al. 2012), behavioural or mental health problems (Timmermans, van Lier et al. 2010) and obesity in children (Gundersen, Mahatmya et al. 2011).

The relationship between psychosocial stress and childhood obesity has been described both cross-sectionally and longitudinally and is hypothesized to result from direct and indirect pathways. Firstly, direct metabolic changes (such as increased visceral fat disposition and a stimulation of appetite) are mainly caused by a dysregulation of the stress system and the production of stress hormones (mainly cortisol and catecholamine) (Timmermans, van Lier et al. 2010, Pervanidou and Chrousos 2011). Secondly, stress may indirectly influence the development of obesity due to behavioural pathways such as maladaptive coping behaviours leading to emotional eating, inactivity and disordered sleep (Dallman, Pecoraro et al. 2003, Akerstedt 2006, Tsatsoulis and Fountoulakis 2006, Torres and Nowson 2007, Biddle and Asare 2011, Michels, Sioen et al. 2012), possibly mediating this stress-obesity relationship (Gatineau and Dent 2011, Pervanidou and Chrousos 2011). However, there is a need for more focused scientific research into the mechanisms linking psychosocial stress to appetite regulation, energy balance and body composition in humans and more importantly in

children, as children may be particularly vulnerable to the effects of chronic alterations in cortisol secretion influencing brain development, and in the endocrine and metabolic systems.

This cross-sectional study examines the association between psychosocial stress and body composition in young children (5-10 years old). As it is recently hypothesized that psychosocial stress may promote the consumption of so-called comfort foods (rich in sugar and fat), a decreased amount of physical activity and disordered sleep (Tsatsoulis and Fountoulakis 2006, Torres and Nowson 2007, Lam and Ip 2010, Michels, Sioen et al. 2012), this study will also contribute to an unexplored domain by investigating to what extent this stress-obesity association is moderated or mediated by sleep duration, diet (sweet food, fatty food, snacks and fruit & vegetables), physical activity or sedentary lifestyle (screen exposure time) in young children. A particular contribution of this study lies within its stress assessment methodology, using both questionnaires and salivary cortisol measurements.

Methodology

Study participants

The ChiBS survey investigates the association between chronic psychosocial stress and changing body composition in Belgian children over a 2-year follow-up period (2010-2012). Detailed aims, design, methods, population and participation characteristics can be found in chapter 2 "Methodology". Participation numbers for the different measurement modules are presented in Figure 26. For body composition, routine anthropometry was available for all children but the more advanced fat% measurement was only available in a subgroup.

Analyses in this study were limited to children with complete information for the stress-related lifestyle parameters (N=355) as full information on these parameters was necessary to perform the moderator and mediator analyses. This sample of 355 children however additionally decreased in number depending on the studied stress and body composition variable in analyses, as presented in Figure 26.

No differences were observed between the included children and children with missing data on age, stress measures and body composition measurements (parametric and non-parametric T-test; data not shown).

Anthropometric examinations

Age- and sex- specific body mass index (BMI) z-scores were calculated according to the method from Cole (Cole, Bellizzi et al. 2000). Fat% was measured with the advanced air displacement plethysmography technology (BOD POD device). The waist-to-height ratio (WHtR) was calculated as an indicator of central body fat to investigate central adiposity. More details can be found in chapter 2 "Methodology".

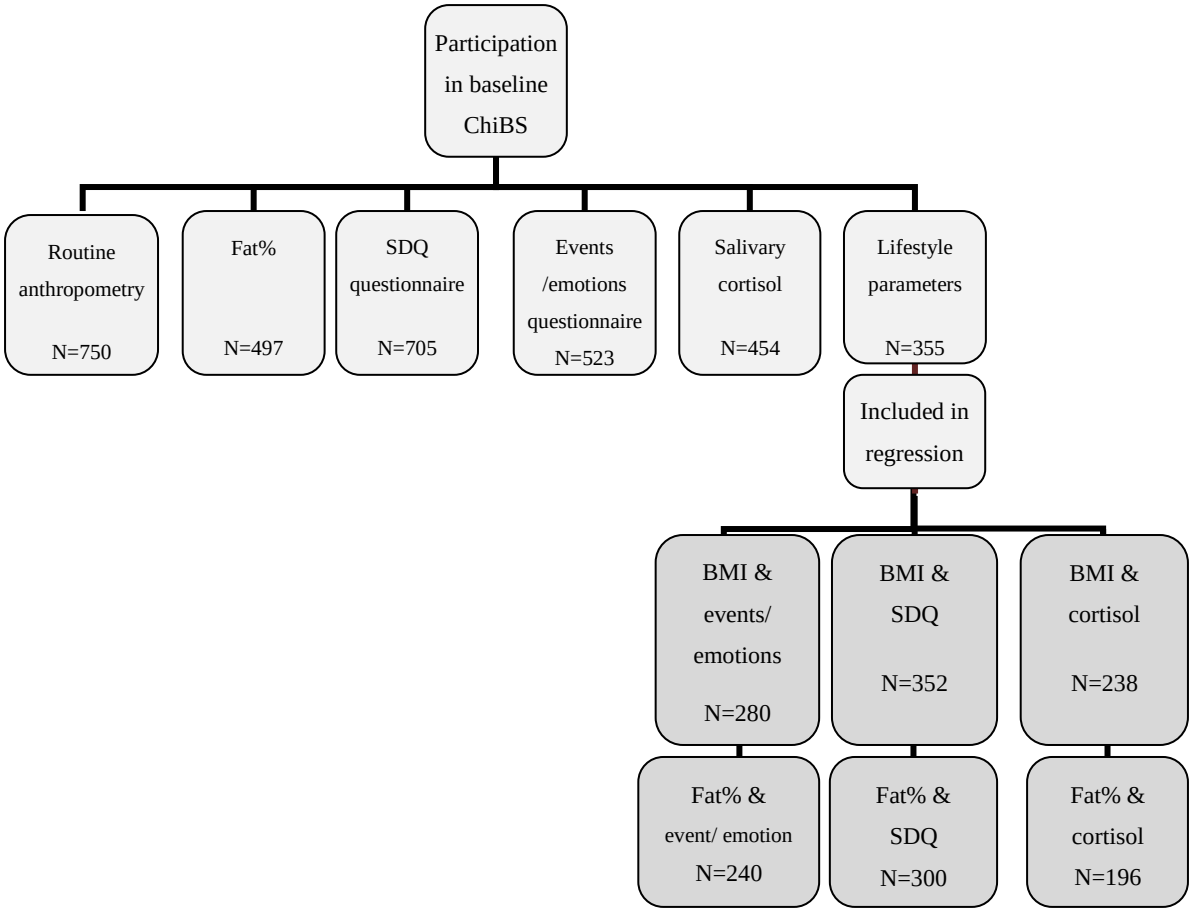


Figure 26: Flowchart

ChiBS= Children's Body Composition and Stress; SDQ= Strengths and Difficulties Questionnaire; BMI= body mass index

Stress parameters

To cover the complete aspect of stress, events, emotions and behavioural problems were examined by questionnaires. A total composite stress score was then also calculated by summing up the z-scores of the three stress aspects per child: one z-score for negative events, one z-score for negative emotions and one z-score for behavioural problems. Furthermore, salivary cortisol was used as an objective stress biomarker.

Life events (child-reported)

The Coddington Life Events Scale for children (CLES-C) assesses the frequency and timing of 36 stressful life events relevant for this age group during the last year (see chapter 2 "Methodology"). For this paper, the negative life events score was calculated for the previous 6 months.

Basic emotions (child-reported)

Children were asked to report on their recent feelings of anger, anxiety, sadness and happiness as described in chapter 2 "Methodology".

Behavioural and emotional problems (parent-reported)

Parents were asked to complete the standardized 'Strengths and Difficulties Questionnaire' (Goodman 1997), reporting children's behavioural and emotional problems over the past 6 months. The statements were divided in four subscales: peer problems, conduct problems, emotional problems and prosocial behaviour. Higher scores on the prosocial behaviour subscale reflect strengths, whereas higher scores on the other three subscales reflect difficulties or problems.

Salivary cortisol analysis

Saliva was collected at home via Salivette synthetic swabs (Sarstedt, Germany) immediately after wake up (T0), 30 minutes after wake up (T30), 60 minutes after wake up (T60) and in the evening between 7 and 9 PM (Tev). More details on sampling and analyses can be found in chapter 2 "Methodology".

Stress-Related Lifestyle Parameters

Information on the children's diet, physical/sedentary behaviour and sleep was collected by a parental-reported questionnaire embedded in the IDEFICS project.

Food consumption frequency

Parents reported on their child's dietary pattern by completing a Food Frequency Questionnaire (FFQ). More information on the FFQ can be found in chapter 2 "Methodology". To identify dietary patterns, four indices on dietary pattern were computed by summing up the frequency of consumption of separate food items. A food index for (1) 'sweet foods' (i.e. sweet drinks, jam, honey, sweet breakfast cereals, sweet snacks), (2) 'fatty

foods' (i.e. fried potatoes, chocolate- or nut-based spreads, high fat dairy, mayonnaise and mayonnaise-based products, cheese, fat meat preparations, butter, high fat snacks), (3) 'unhealthy snacks, including foods that are fat and/or sweet' (i.e. chocolate and chocolate bars, candy, biscuits, cake, ice-cream, chips) and (4) a healthy food index for 'fruit and vegetables' (i.e. fruit, freshly squeezed fruit juice, vegetables) was calculated.

Sleep duration, physical activity and screen exposure time

Parents reported the typical hours of bedtime and getting up in the morning for weekdays, from which the child's sleep duration during the week was calculated. Parents reported also the number of screen time hours (e.g. television and computer time) as a measure of sedentary behaviour and the hours of physical activity (outdoor and at sport clubs).

Possible confounders

Parental education was collected as reported in chapter 2 "Methodology". Children's sex was reported by the parents and children's age was calculated from birth date and examination date.

Statistical Analysis

Analyses were performed using PASW Statistical Program version 19.0 (SPSS Inc, IBM, IL, USA). The two-sided level of significance was set at $p<0.05$. Data were presented by their median and interquartile range to handle non-normally distributed data. The difference between boys and girls was examined for continuous variables (Independent Samples *T*-test and Mann-Whitney *U* Test for normally distributed data and skewed data respectively) and for categorical variables (Pearson Chi-Square Test). The association between stress and body composition was analysed using linear regression analyses. All regression analyses were adjusted for sex, age and parental education as sex and age are significantly associated with body composition measures (age: ß= 0.204, $p<0.001$ and ß= -0.282, $p<0.001$ for BMI and WHtR respectively) (sex: ß=0.093, $p=0.077$; ß=0.340, $p<0.001$ and ß=0.125, $p=0.015$ for BMI, fat% and WHtR respectively) and parental education is a known factor in influencing children's lifestyle and behaviour. Analyses were only performed for girls and boys separately if a significant sex interaction factor was present. The distributions of WHtR, consumption frequencies, screen time and sleep duration were skewed and were therefore logarithmically transformed. The regression residuals were normally distributed.

A first set of regressions tested the direct and indirect association between stress (predictor variable) and body composition (outcome variable) and examined the mediating role of lifestyle parameters, as well as sex differences in this association. Mediation was tested according to Baron and Kenny (Baron and Kenny 1986). In doing this, the significant reduction of the association between the stress parameter (independent variable) and body composition (dependent variable) after controlling for lifestyle (mediator) was tested non-parametrically by bootstrapping (using 10 000 samples) (Preacher and Hayes 2004).

A second set of regressions tested moderation by sex and lifestyle parameters in the association between stress and body composition. According to literature, moderation was tested by including an interaction factor (Frazier, Tix et al. 2004). In those regressions, continuous parameters were transformed in z-scores and the categorical sex variable was effect coded (-1 and 1 for boys and girls respectively). A $p<0.10$ was used as indicator for moderation as the power to detect interaction effects is lower than for main effects (Frazier, Tix et al. 2004). If significant, visual representation was done by plotting predicted outcome values (based on the non-standardised coefficients) for three representative groups of the moderator: those at the mean, at one SD below the mean and one SD above the mean. Statistical interpretation was done by testing the significance of the stress predictor for the two groups: for sex, this was boys and girls; for the continuous moderator (the lifestyle parameters) two groups were created based on a median split. If the predictor was significant in a certain subgroup, the beta and significance values are reported.

Results

Participant characteristics

Table 33 describes the studied anthropometrical, stress and lifestyle parameters for the participating boys and girls separately. Boys and girls did differ in the following studied parameters 1) conduct problems, which were significantly higher in boys; 2) prosocial behaviour which was significantly higher in girls, 3) physical activity, which was higher in boys and 4) fat% and overweight/obesity prevalence were significantly higher in girls.

Table 33: Participant characteristics (N=355)

	Boys (N=186)			Girls (N=169)			
	Median	**P25**	**P75**	**Median**	**P25**	**P75**	**p-value**
Age (years)	8.00	6.80	9.20	8.00	6.75	9.00	0.475[#]
Body composition parameters							
BMI z-score	-0.15	-0.74	0.37	-0.11	-0.73	0.66	0.222[#]
Fat%	18.48	15.44	22.10	21.96	18.76	26.91	**<0.001**
Waist-to-height ratio	0.43	0.41	0.45	0.44	0.42	0.46	0.064
Stress parameters							
Negative events 0-6m	21	0	52	29	0	53	0.476
Happiness (scale 0-10)	8	6	10	8	6	9	0.675
Anxiety (scale 0-10)	1	0	3	1	0	4	0.155
Sadness (scale 0-10)	2	0	4	2	1	5	0.078
Anger (scale 0-10)	2	1	4	3	1	5	0.074
Peer problems (scale 0-10)	1	0	2	1	0	2	0.945
Conduct problems (scale 0-10)	1	0	2	1	0	2	**0.048**
Emotional problems (scale 0-10)	2	1	3	2	1	4	0.251
Prosocial behaviour (scale 0-10)	7	5	8	7	6	8	**0.044**
Salivary cortisol awakening (nmol/l)	11.89	10.09	13.93	11.33	9.51	14.71	0.828
Salivary cortisol AUCg	23.09	17.57	27.54	23.27	17.83	28.39	0.617
Salivary cortisol decline	-0.78	-0.96	-0.58	-0.76	-1.01	-0.62	0.272
Stress-related lifestyle parameters							
Sleep duration (hours on weekdays)	11.0	10.5	11.2	11.0	10.5	11.5	0.585
High-caloric snack frequency (times/week)	8.5	5.0	13.2	9.0	6.0	13.0	0.284
Sweet food frequency (times/week)	30.0	22.5	40.5	28.5	19.0	38.0	0.054
Fatty food frequency (times/week)	25.0	19.0	34.0	25.0	19.0	35.0	0.778
Fruit and vegetables frequency (times/week)	16.0	11.0	18.0	14.00	11.0	19.0	0.859
Physical activity (hours/week)	15.00	10.50	20.75	13.50	9.00	19.00	**0.004**
Screen time (hours/week)	11.50	7.25	15.50	10.25	7.00	14.00	0.051

	N (%)	N (%)	
BMI categories (Cole)			
Underweight	26 (14%)	20 (12%)	**0.021**[$]
Normal weight	152 (82%)	132 (78%)	
Overweight and obese	8 (4%)	17 (10%)	
Maximal parental education			
ISCED level 2 and 3	50 (27%)	58 (35%)	0.271[$]
ISCED level 4	41 (22%)	31 (18%)	
ISCED level 5	95 (51%)	80 (47%)	

p-value of Mann-Whitney U Test
[#] p-value of Independent Samples T-test
[$]p-value of Pearson Chi-Square Test
ISCED= International Standard Classification of Education, 2 'lower secondary education', 3 'upper secondary education', 4 'post-secondary non-tertiary education', 5 'first stage of tertiary education'

Stress – body composition association and its mediation by stress-related lifestyle parameters

The association between stress and body composition was studied directly and indirectly through the possible mediating role of stress-related lifestyle parameters. Table 34 presents the significant results of the linear regression models investigating the association between stress and body composition, with and without adjustment for the studied lifestyle factors. The adjusted linear regression models were repeated with another snack index, i.e. including soft drinks, and resulted in similar results as described below (data not shown).

Neither a direct nor an indirect association was observed between the following stress parameters and body composition: anger, anxiety, sadness, conduct problems, emotional problems, prosocial behaviour, salivary awakening cortisol, salivary AUCg cortisol and salivary decline (data not shown). However, a positive association (both directly (unadjusted β's) and independently (adjusted β's)) was shown for children's BMI z-score and WHtR with the negative events score for the past 6 months, while an inverse association was seen with happiness (Table 34). The composite stress score was also positively related to WHtR. A sex interaction was observed in the association of peer problems with WHtR and fat%: this association was only significant in girls (Figure 27).

Table 34: The significant stress-body composition association: results of multivariate linear regression models, unadjusted and adjusted for lifestyle.

	OUTCOME					
	BMI z-score		**WHtR**		**Fat%**	
	ß	**p**	**ß**	**p**	**ß**	**p**
PREDICTOR						
negative events 0-6m						
unadjusted	0.129	0.033	0.155	0.009		
adjusted	0.116	0.050	0.147	0.013		
happiness						
unadjusted	-0.134	0.027	-0.143	0.017		
adjusted	-0.120	0.044	-0.148	0.014		
composite stress score						
unadjusted			0.134	0.011		
adjusted			0.142	0.022		
peer problems (girls only)						
unadjusted			0.156	0.042	0.169	0.011
adjusted			0.154	0.044	0.155	0.068

All analyses were adjusted for age, sex and parental education. Only significant stress-body composition associations are presented.

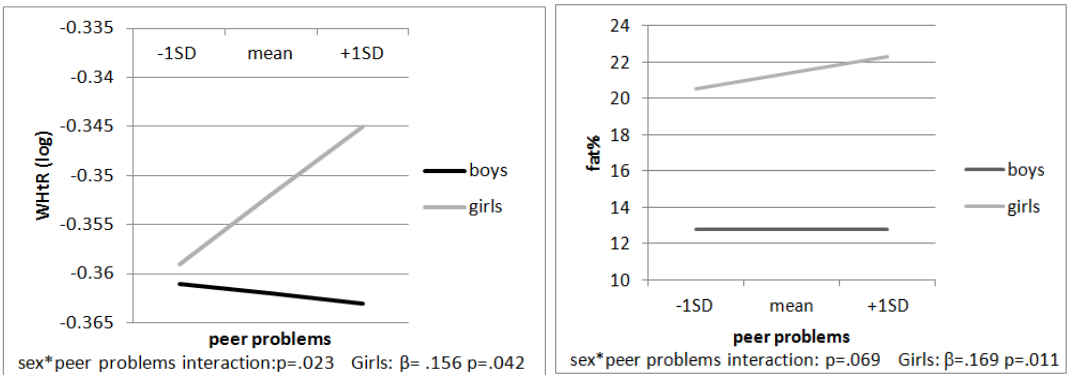


Figure 27 : Sex as moderator in the stress – body composition relation

The stress – body composition relation are shown for boys and girls and for stress levels equal to the mean, one standard deviation above the mean (=high) or one standard deviation below the mean (=low). The significance value of the interaction term for sex - stress in the relation with body composition is given. If the stress – body composition relation was significant in one of the sexes, those significance and bèta values are also given.

After correction for screen time (in the association between peer problems and WHtR in girls) and after correction for high-caloric snack index (in the association between negative events 0-6m and BMI z-score) the stress – body composition association was no longer significant (data not shown), indicating a possible mediating role of these lifestyle factors. However, bootstrapping showed that the stress-overweight association was not significantly reduced after correction for these lifestyle factors (indirect confidence interval [-0.0002; 0.0015] and [-0.0004; 0.0012], respectively), demonstrating that these lifestyle factors do not mediate the stress-obesity association and that stress may be an independent risk factor for obesity.

Moderation by stress-related lifestyle factors

The studied lifestyle parameters were also tested as moderators in the association between stress and body composition, as graphically presented in Figure 28. Sleep duration, snack consumption frequency; sweet food consumption frequency and screen time were found to moderate one or more of the stress – body composition associations. Fatty food consumption frequency, fruit & vegetable consumption frequency and physical activity did not moderate stress – body composition associations.

More specifically, the positive association between stress (negative events, peer problems, cortisol morning levels and cortisol decline) and body composition (BMI, fat% and WHtR) was strengthened by unhealthy behaviour (high-caloric snack frequency, sweet food frequency, high screen time, low sleep duration), e.g. stress as presented by a large amount of

negative events was highly related to a higher BMI z-score in the presence of the unhealthy behaviour of high-caloric snack intake frequency (similar results for snack intake including soft drinks) while almost no association between stress and body composition was found for the healthy behaviour of low snack intake frequency. Nevertheless, the sleep moderation for sadness was reversed: a positive association between stress and body composition was only seen in children with high sleep duration.

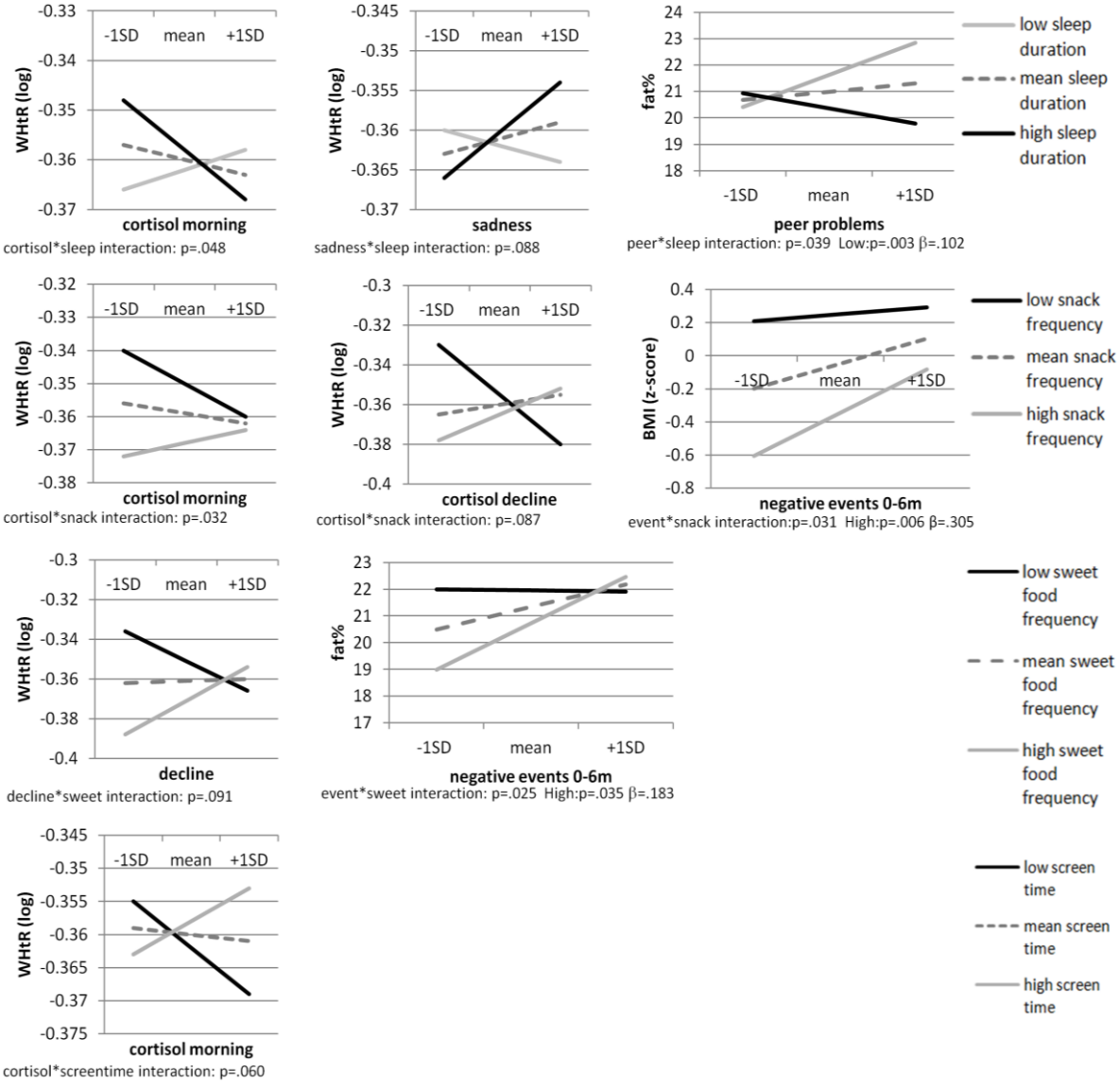


Figure 28: Lifestyle factors (sleep duration, high-caloric snack frequency, sweet food frequency and screen time) as moderator in the stress – body composition relation

Figures show the stress – body composition relation for moderator and stress levels equal to the mean, 1 standard deviation above the mean (=high) or 1 standard deviation below the mean (=low). The significance value of the interaction term for lifestyle - stress is given. If the stress – body composition relation was significant in one of the two subgroups of the moderator (groups with score above or below the mean of the lifestyle factor), those significance and bèta values are also given.

Discussion

This study examined the cross-sectional association between psychosocial stress and body composition in young children and investigated the role of sleep, diet, physical activity and screen time as lifestyle factors mediating or moderating this association.

The main findings of this study were (1) the observed positive association between high stress (mainly negative life events, low happiness and peer problems) and body composition measures in a direct way, (2) the lack of mediation effects for the studied lifestyle factors (i.e. the lifestyle factors did not account for the observed stress-body composition association) and (3) the observation of some moderation: the stress-body composition association was strengthened by the most unhealthy lifestyle patterns (i.e. lowest sleep duration, highest snack consumption, highest sweet food consumption or highest screen time).

To our knowledge, this study was the first examining the association between stress and body composition in young children by using multiple stress measures, standardized body composition measurements and by incorporating stress-related lifestyle factors. That is why comparisons of our findings with observations from similar studies were limited. Nevertheless, our general findings on stress-body composition are in agreement with previous studies indicating an association between stress and obesity/body composition in children: Gundersen et al. (Gundersen, Mahatmya et al. 2011) concluded that in all studies on psychosocial stress and childhood obesity at least one measure of stress was associated with obesity. Also in our study, not all stress measures related to the body composition measures: (1) Only negative life events, low happiness and peer problems (the latter only in girls) related to body composition; while in the moderation analyses also other stress measures (such as the cortisol measures) were related; (2) No direct significant association was observed between cortisol and body composition, indicating the importance of child-reported stress measures in the stress-body composition association. These observations could point to specificity in the stress-body composition association (i.e. unique, specific relation between a particular risk factor and outcome) (McMahon, Grant et al. 2003). However, our study was not designed to study specificity in the stress-body composition association.

More importantly, in our study both overall (BMI z-score, fat%) and central (WHtR) obesity parameters were related to stress parameters (i.e. life events, happiness and/or peer problems). The composite stress score was only related to higher central adiposity (WHtR). Indeed, stress

has been shown to predominantly stimulate abdominal accumulation of fat since the glucocorticoid receptors (by which cortisol exerts its fat deposition effect) have a high density in the abdominal fat (Bjorntorp 2001). A final specificity in the relation was seen with sex i.e. a significant relation of peer problems with fat% and WHtR in girls only. Also previously, sex differences in the stress-related hormonal pathways have been shown (Bjorntorp 2001). A possible explanation in this preadolescent population could be a sex difference in children's experiences of peer interactions as it has recently been reviewed that girls are more sensitive to their friendship status and to receiving higher levels of emotional provisions in their friendships (Rose and Rudolph 2006). Indeed, sex has been mentioned as a possible moderator on stress and mental health in children (Gatineau and Dent 2011).

Another research aim was to examine possible underlying pathways in this stress-body composition association. From a physiological point of view, we expected that the association between stress and body composition would operate through the energy balance, i.e. through influencing energy intake (i.e. diet) or energy expenditure (e.g. screen time and physical activity). In previous research, the lifestyle factors diet, activity/sedentary lifestyle and sleep have been related to stress and more consistently to childhood obesity (Dallman, Pecoraro et al. 2003, Akerstedt 2006, Torres and Nowson 2007, Holmes, Ekkekakis et al. 2010, Biddle and Asare 2011). Testing moderating or mediating effects of lifestyle in the association between stress or mental health with obesity is a largely unexplored domain (Gatineau and Dent 2011, Pervanidou and Chrousos 2011). In a recent review, low physical activity and an unhealthy diet were mentioned as mediators for the effect of mental health on obesity, while sleep was hypothesised as a mediator in the effect of obesity on mental health. That review also proposed physical activity as mediator regarding the impact of mental health on children's obesity, but not a mediating effect for the other lifestyle factors (Gatineau and Dent 2011). In a recent stress-obesity study examining all mentioned lifestyle factors in adolescents, stress was related to general and central adiposity, an unhealthier diet and a shorter sleep duration on weekdays but they found no mediating effect of these lifestyle factors on the stress-obesity relation (De Vriendt, Moreno et al. 2009), which is in line with our study: no mediating effects of lifestyle factors were observed. This may either point to the importance of non-behavioural pathways in the development of overweight/obesity (e.g. stress-induced hormonal changes causing increased fat deposition), or this may be explained by (1) the cross-sectional design of this study potentially not allowing to observe mediation pathways, or to (2) methodological limitations in the chosen lifestyle variables.

Although the studied lifestyle factors have not been observed as mediators, they acted as moderators of the stress-body composition association. In literature, we have indeed found studies showing e.g. children's physical activity as moderator in the association between stress and overweight or metabolic risk (Yin, Davis et al. 2005, Holmes, Ekkekakis et al. 2010). Consequently, children with an unhealthier lifestyle could be more vulnerable for stress-induced overweight or, vice versa, a healthy life style could be a buffer for the effects of stress on adiposity. Especially in the presence of low sleep duration or high consumption frequency of high-caloric snacks and sweet foods, the positive association between stress and body composition was strengthened in our study. To a lesser degree, this was also seen for sedentary behaviour. This was not seen for the other diet parameters and physical activity. On the contrary, a moderating effect of sleep on the association between sadness and overweight was observed in an unexpected direction, i.e. the stress-body composition association was strengthened in children with a long sleep duration, which is surprising since in particular sleep deprivation has been associated with obesity. Although we have no clear explanation for this finding, it is possible that children feeling sad have the need to sleep longer. Last, it is assumed that lifestyle may be the underlying mechanism in the stress-obesity association, although from another perspective it is possible that psychosocial stress is clustered with the exposure to an unhealthy lifestyle (e.g. low SES and unhealthy diet patterns), and thereby both have an impact on obesity.

Strengths and limitations

To our knowledge, this study was the first to examine the association between stress and body composition in young children by using more than one stress measure (stressor questionnaires, salivary cortisol) and standardized measurements of overall and central adiposity (BMI, WHtR and the more advanced fat%) and by considering more than one stress-related lifestyle factor, which are the main strong methodological features of this study. Some limitations should however be considered when interpreting the results. Firstly, we acknowledge the cross-sectional design of this study to be its largest weakness in not allowing to study causality. However, as the ChiBS project progresses, the association between stress and changes in body composition will be further investigated on a longitudinal basis. Also, the directionality of the association between mental health and body composition could be different for youth and adults (Napolitano and Foster 2008). Secondly, the fat% measurements with the BOD POD were only available in a smaller sample size compared to the routine anthropometric measurements (BMI and WHtR) because of the additional effort

asked from parents in transporting their children to another survey centre where the BODPOD was located. Thirdly, a reporting bias in the parent-reported lifestyle parameters (parents may have the tendency to give the morally right answer or may inaccurately estimate their child's lifestyle behaviour) and in the child-reported stress questionnaires (i.e. CLES and basic emotions) cannot be excluded, as a result of which reversed causation may be possible. Concerning the studied lifestyle parameters, methodologically more profound measurements were available in the ChiBS project such as accelerometry/actigraphy for physical activity and sleep quality, but were not applied in this study because of power issues (i.e. low sample sizes). In this context, it should be noted that the measurement of sleep duration covers the numbers of hours spent in bed and not necessarily the amount of sleep. Next, the FFQ did not include portion sizes and therefore, no energy intake could be calculated (Rodriguez and Moreno 2006). Nevertheless, using a FFQ has the advantage of showing the habitual diet as dietary recalls can be biased by exceptional days. Lastly, as the participating children were characterized by a high socio-economic background, we may have missed stronger associations between stress and body composition in lower socio-economic environments. However, no additional selection bias was introduced by including only those children with complete information for the studied variables (as discussed in the "methodology" section).

Conclusion

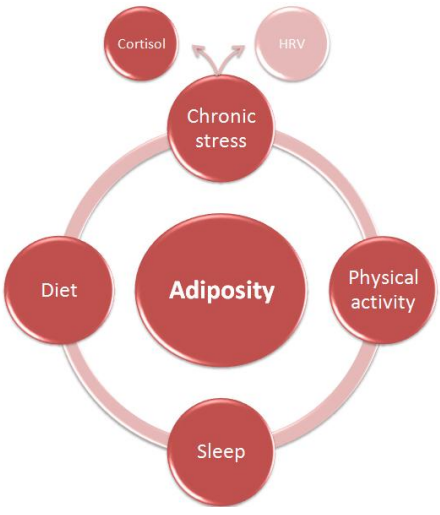
Children's stress level, characterized by negative life events, low happiness and peer problems, was associated with overall and central adiposity. Lifestyle factors (i.e. sleep duration, high-caloric snacking, sweet food consumption and screen time) did not mediate this relationship, although they were shown to intervene as moderators. Consequently, an unhealthy lifestyle may make children more prone to stress-induced changes in body composition or, vice versa, a healthy lifestyle may attenuate the effects of stress on adiposity. When framing these results in a public health perspective, it may be recommended to screen for the presence of psychosocial stress and to incorporate education on stress management in obesity prevention programs. Moreover, the moderating effects of lifestyle factors put stress into a new perspective and indicate the importance of multi-factorial obesity prevention programs by focusing concurrently on several lifestyle factors.

2.

Cross-lagged associations between children's stress and adiposity: the ChiBS study

Michels N, Sioen I, Boone L, Clays E, Vanaelst B, Huybrechts I, De Henauw S

Psychosomatic Medicine, submitted.



Stress is an important dragon to slay

– or at least tame – in your life.

ABSTRACT

INTRODUCTION: Stress and adiposity are important threats for public health that have been associated with each other. Longitudinal studies are needed to reveal the (bi)directionality of the relation and the underlying factors, especially in young children. We hypothesize that stress might increase adiposity by hormonal changes (high cortisol) or by deteriorating lifestyle.

METHODS: In 326 Belgian children (5-12y) of the ChiBS study, the longitudinal relation between stress and adiposity was examined over two years. Stress was reported by questionnaires concerning negative life events, problem behaviour and emotions. BMI, waist-to-height and fat% estimated by air displacement were used as adiposity parameters. Stress reactivity was measured by salivary cortisol (4 samples/day, 2 days). Furthermore, physical activity, screen time, food consumption, eating behaviour and sleep duration were measured as lifestyle factors. Cross-lagged analyses with Mplus examined bidirectional longitudinal stress-adiposity relations. Moderation and mediation by cortisol and lifestyle was tested in the effects of stress on adiposity.

RESULTS: Cross-lagged analyses showed no longitudinal effect of stress on adiposity, but adiposity (BMI and fat%) increased stress. Nevertheless, stress influenced adiposity after moderation by cortisol and lifestyle: stress increased adiposity in those with high cortisol patterns, high sweet food consumption and long screen time, while stress decreased adiposity in the most active children (short sedentary time, long physical activity time).

DISCUSSION: Depending on cortisol levels and lifestyle, stress affects children's adiposity. Stress increased adiposity in children with an unhealthy lifestyle and stress decreased adiposity in those with a healthy, active lifestyle. This creates a perspective for multi-factorial obesity prevention, targeting stress and lifestyle factors in parallel. Moreover, our results also highlight the adverse effect of an unhealthy body composition on children's psychological health.

Introduction

The last decades have been characterized by a global increasing overweight epidemic. Most alarming is the rise in childhood overweight with prevalence ranging between 10 and 40% in European countries (Moreno, Pigeot et al. 2011). Research has broadened its view on potential obesity contributing factors that extend beyond the traditional concept of energy imbalance by diet and physical activity. In this context, especially the psychological determinants of obesity have received increasing interest (Rehkopf, Laraia et al. 2011, Karasu 2012). Recent meta-analyses in adults (Luppino, de Wit et al. 2010, Wardle, Chida et al. 2011) and also some reviews in children/adolescents (Liem, Sauer et al. 2008, Gundersen, Mahatmya et al. 2011, Incledon, Wake et al. 2011) have mainly uncovered an adiposity increasing effect of stress (stressors, perceived stress, depression, anxiety and behavioural problems), although also null effects and even opposite results were sometimes observed. Interestingly, the association might be bidirectional with adiposity also influencing stress levels (Luppino, de Wit et al. 2010, Foss and Dyrstad 2011, Gatineau and Dent 2011).

The effects of psychosocial stress on childhood adiposity have been hypothesized to result from direct and indirect pathways. Firstly, direct metabolic changes (such as increased visceral fat disposition and a stimulation of appetite) are mainly caused by a dysregulation of the stress system and stress hormone production (e.g. cortisol and catecholamines) (Bjorntorp 2001, Charmandari, Tsigos et al. 2005, Pervanidou and Chrousos 2011). Secondly, stress may indirectly facilitate adiposity through behavioural pathways: stress may change the energy balance by interrupting a child's choice to participate in healthy behaviours such as physical activity and adequate sleep duration, while increasing unhealthy behaviours such as emotional eating of unhealthy food items and sedentary screen time (Dallman, Pecoraro et al. 2003, Buckley and Schatzberg 2005, Akerstedt 2006, Tsatsoulis and Fountoulakis 2006, Adam and Epel 2007, Kappos 2007, Torres and Nowson 2007, Biddle and Asare 2011, Balantekin and Roemmich 2012, Epel, Tomiyama et al. 2012). Nevertheless, little evidence is available for these underlying endocrinological and behavioural factors of the stress effect (Gatineau and Dent 2011).

Overall, the literature recommends further research with longitudinal designs, using accurately measured body composition and examining the underlying endocrinological and behavioural factors (Wilson and Sato 2013). Moreover, there is a lack of evidence for this relation in the young age groups of primary school children (Gatineau and Dent 2011,

Incledon, Wake et al. 2011). Since adult psychopathology (Fryers and Brugha 2013), lifestyle (Thorleifsdottir, Bjornsson et al. 2002, Mikkila, Rasanen et al. 2005, Ashcroft, Semmler et al. 2008, Telama 2009) and adiposity (Singh, Mulder et al. 2008) can all find their first foundations in childhood, research should also focus on children. After all, chronic alterations in stress activity may have permanent effects on endocrine and metabolic systems (Teicher, Andersen et al. 2002, McEwen 2008).

Based on these literature findings, the current study aims to examine the bidirectional longitudinal association between stress and adiposity (body mass index, waist and accurate fat% measurements) in Belgian primary school children (5-12y old). A second aim was to test moderation and mediation in the stress effects on adiposity by lifestyle parameters (physical/sedentary activity, diet, eating behaviour and sleep duration) and cortisol levels.

Methods

Design

Participants were Belgian children (50% boys) recruited for the longitudinal ChiBS study (2010-2012). The children were measured during 3 waves (i.e. measurement periods) with 1-year interval: in 2010, 2011 and 2012. At baseline, children were between 5 and 10 years old. More details on the ChiBS study can be found in chapter 2 “Methodology”.

Overall, 523 children participated in 2010 (T0), 455 in 2011 (T1) and 330 in 2012 (T2). We included 326 children that participated in all 3 waves and an extra 129 children in only 2 waves. Participants with and without complete data were compared using Little’s Missing Completely At Random test (Little 1988). A non-significant χ^2 test statistic suggests that missing data are missing only in a random way and hence do not introduce any bias with regard to the central research question. No differences between the follow-up and drop-out population was seen on stress, adiposity and tested moderators, except for a higher screen time in those that dropped-out ($p=0.016$). Most measurements were performed in all three waves, but only two wave data were available for sleep (2010, 2012), accelerometry (2010, 2012), eating behaviour (2011, 2012) and waist-to-height ratio (2010, 2012). Salivary cortisol data were only measured in the first wave.

Reported stress (T0, T1, T2)

To cover the complete aspect of stress, negative events, emotions and behavioural problems were examined by questionnaires. The three different stress aspects were studied separately.

A total composite stress score (CSS) was then also calculated by summing up the z-scores of the three stress aspects per child: one z-score for negative events, one z-score for negative emotions and one z-score for behavioural problems.

Negative events (child-reported)

The Coddington Life Events Scale for children (CLES-C) assesses the frequency and timing of 36 stressful life events relevant for this age group during the last year (see chapter 2 "Methodology"). The score of negative life events was used.

Negative emotions (child-reported)

Children had to indicate how the mostly feel as described in chapter 2 "Methodology".

Behavioural problems (parent-reported)

Parents were asked to complete the standardized 'Strengths and Difficulties Questionnaire' (SDQ) (Goodman 1997), reporting children's behavioural problems over the past 6 months. The statements were divided in subscales with higher scores reflecting difficulties or problems: peer problems, conduct problems and emotional problems.

Salivary cortisol for stress reactivity (only T0)

Saliva was collected at home via Salivette synthetic swabs (Sarstedt, Germany) immediately after wake up (T0), 30 minutes after wake up (T30), 60 minutes after wake up (T60) and in the evening (Tev). More details on sampling and analyses can be found in chapter 2 "Methodology". Apart from single-point cortisol concentrations, also summary variables have been calculated to represent two independent cortisol patterns over time: the cortisol awakening response and the diurnal decline (Pruessner, Kirschbaum et al. 2003, Fekedulegn, Andrew et al. 2007). To represent the cortisol awakening response, the area under the curve with respect to the ground (AUCg) and the area under the curve with respect to increase (AUCi) were calculated between the T0 and T60 sample. These two parameters can reveal different information. The AUCi measures the pure morning increase, reflecting the sensitivity of the cortisol axis and has been more related to stress perception. The AUCg also measures the morning increase but taking into account the basal level, reflecting the total hormonal output and has more been related to physical complaints. The diurnal cortisol decline was investigated as the concentration of T0 minus Tev, divided by the number of hours between these sampling periods, with a more positive value representing a steeper

decline. Higher cortisol levels and a steeper diurnal decline have been related to more stress in our population (Michels, Sioen et al. 2012).

Adiposity (T0, T1, T2 but WHtR only T0 and T2)

Adiposity was measured by age- and sex- specific BMI z-scores, fat percentage (fat%) using air-displacement plethysmography (BOD POD®) and the waist-to-height ratio (WHtR) as described in chapter 2 "Methodology". While BMI and fat% reflect overall adiposity, the WHtR is an indicator of central body fat.

Lifestyle factors

Physical activity and sedentary behaviour (parent-reported at T0, T1 and T2)

Parents were asked about the usual physical activity and screen time of their child at each measurement wave. The sum of usual hours of physical activity outdoors and at sports club per week were used as a measure of physical activity. The reported usual number of screen time hours per week (e.g. television and computer time) was used as a measure of sedentary behaviour in the analyses.

Physical activity and sedentary behaviour (objectively measured at T0 and T2)

Physical activity was also measured with accelerometers for at least 3 days in 2010 and 2012 as described in chapter 2 "Methodology". Sedentary and moderate-to-vigorous physical activity were detected using the cut-off points of Evenson and expressed using percentages to correct for wearing time.

Food Frequency Questionnaire (FFQ) (parent-reported at T0, T1 and T2)

Parents reported on their child's dietary pattern during the last 4 weeks by completing the FFQ. More information on the FFQ can be found in chapter 2 "Methodology". To identify dietary patterns, four indices on dietary pattern were computed by summing up the frequency of consumption of separate food items. A food index for (1) 'sweet foods' (i.e. sweet drinks, jam, honey, sweet breakfast cereals, sweet snacks), (2) 'fatty foods' (i.e. fried potatoes, chocolate- or nut-based spreads, high fat dairy, mayonnaise and mayonnaise-based products, cheese, fat meat preparations, butter, high fat snacks), (3) 'unhealthy snacks, including foods that are fat and/or sweet' (i.e. chocolate and chocolate bars, candy, biscuits, cake, ice-cream, chips) and (4) a healthy food index for 'fruit and vegetables' (i.e. fruit, freshly squeezed fruit juice, vegetables) was calculated.

Dutch Eating Behaviour Questionnaire (DEBQ) (child-reported at T1 and T2)

Children had to fill in the 33-item DEBQ on their usual eating behaviour. Three types of eating behaviour can be identified in children: eating in response to negative emotions (emotional eating), eating in response to the sight or smell of food (external eating) and eating less than desired to lose or maintain body weight (restrained eating). In all three types of eating behaviour, the appropriate self-regulating mechanism of food intake is diminished or lost.

Sleep (parent-reported at T0 and T2)

Parents reported the typical hours of bedtime and getting up in the morning for weekdays and weekend days, from which the child's sleep duration was calculated.

Possible confounders

Sex, age and socio-economic status were considered as potential confounding factors. The children's sex and birth date were reported by the parent. Parental education (to represent socio-economic status) was assessed by questionnaire according to the International Standard Classification of Education.

Statistics

Cross-lagged stress-adiposity relations

Structural equation modelling (SEM) in Mplus (version 5.1) with maximum-likelihood estimation was used to determine longitudinal associations between stress (the CSS, negative events, negative emotions and total problems) and adiposity (BMI, fat% and WHtR). A significance level of $p<0.05$ was used. A number of fit indices were used to evaluate the model: χ^2 test, the comparative fit index (CFI) and the root mean square error of approximation (RMSEA). χ^2 /df ratio of 2 or below, CFI values of 0.90 or above and RMSEA values of 0.06 or below were used as indicators of acceptable fit (Kline 2005).

Measurement model To correct for measurement error, three parcels were created in modeling total problems (using the subscales peer problems, conduct problems, emotional problems) and negative emotions (using the subscales anger, anxiety, sadness). Indicators had significant and moderate to strong loadings on the latent factors, ranging from 0.44 to 0.91 (all $p<0.001$). Measurement models demonstrated a good fit to the data.

Structural model Cross-lagged models were used, including 1) cross-lagged paths (e.g., from stress at baseline to adiposity at follow-up); 2) autoregressive paths (e.g., stress at time 1 to stress at time 2); 3) correlations within waves. To control for possible confounders (age, sex and socio-economic status), paths were allowed from each of these three variables to all the constructs included in the structural models.

Multi-group analysis Multi-group comparisons were tested to investigate potential structural differences for the cross-lagged models depending on sex and age. Age was transformed in a categorical variable by creating two groups based on a median (one group <8, one group ≥8). This analysis was done by comparing the fit between the constrained model, in which the structural relations between both groups were not allowed to vary and the unconstrained model, in which the structural relations were set free. The difference in chi-square statistic and CFI statistic between both models was calculated as follows: "constrained model statistic – unconstrained model statistic". Non-equivalence between groups was considered in the case of a significant χ^2 difference and a CFI difference higher than 0.01.

Cortisol and lifestyle as moderators or mediators in the effects of stress on adiposity.

Moderation A moderator is a third variable affecting the direction and/or strength of the relationship between a predictor and outcome variable. Moderation by lifestyle factors (food indices, eating behaviour, physical and sedentary behaviour) and salivary cortisol (AUCg, AUCi and diurnal decline) in the effects of stress on adiposity was tested by including an interaction term between the predictor and the possible moderator.

Mediation Mediation is defined as a variable carrying the influence of a predictor to a given outcome (an intermediate pathway), and thus accounting for the observed relationship. We addressed our hypotheses in three steps (the direct effects, a full mediation and a partial mediation), thereby following the guidelines of Holmbeck (Holmbeck 1997).

Results

Descriptive characteristics

Descriptive data of the stress, lifestyle and adiposity parameters are shown in Table 35. The overweight prevalence in this cohort was around 8% (following the International Obesity Task Force classification). A significant increase in negative events and problems and a significant

decrease in all three negative emotions was seen between 2010 and 2012. Changes were also seen in adiposity: increased BMI z-scores, fat% and WHtR.

Table 35: Descriptive data of stress and adiposity parameters at baseline and second follow-up

	2010			2012			p-value*	N
	P25	P50	P75	P25	P50	P75		
Stress questionnaire data								
Negative events (score)	10	40	73	24	63	106	<0.001	264
Negative emotions (0-30)	3	7	11	2	5	9	<0.001	265
anger (0-10)	1	3	5	1	2	3	0.006	265
anxiety (0-10)	0	1	3	0	0	2	0.004	265
sadness (0-10)	0	2	4	0	1	4	<0.001	265
SDQ: total problems (0-30)	2	4	7	3	4	7	<0.001	315
conduct problems (0-10)	0	1	2	0	2	3	0.004	315
peer problems (0-10)	0	1	2	0	1	2	0.076	316
emotional problems (0-10)	1	2	3	1	2	3	0.565	316
Adiposity								
Body mass index z-score	-0.82	-0.14	0.46	-0.86	-0.21	0.49	0.016	328
Body fat percentage	14.8	18.5	23.6	15.4	19.7	24.5	0.003	305
Waist-to-height ratio	0.42	0.44	0.46	0.48	0.50	0.52	<0.001	325

** Wilcoxon signed-rank test p-value for individual change over 2010-2012; P25/50/75: 25th, 50th and 75th percentile*

Cross-lagged models

Cross-lagged analyses showed that adiposity (BMI and fat%) could influence the overall stress level over time (see Figure 29). Nevertheless, the models could not confirm that stress influences adiposity longitudinally (p>0.05). Significances remained when only allowing unidirectional relations. To examine which specific stress aspects were responsible for this

relation, the cross-lagged analyses were repeated on the separate stress items: similar results were found for negative emotions. Since the effect of adiposity on stress could conceal a possible effect of stress on adiposity, the analyses were repeated on only those that were not overweight at baseline, but this did not change our results. Multi-group analyses showed that the models were not different for boys and girls (χ^2 difference p>0.05 and CFI difference <0.01) but that the models with the problem score were different between the younger and older children (Table 36).

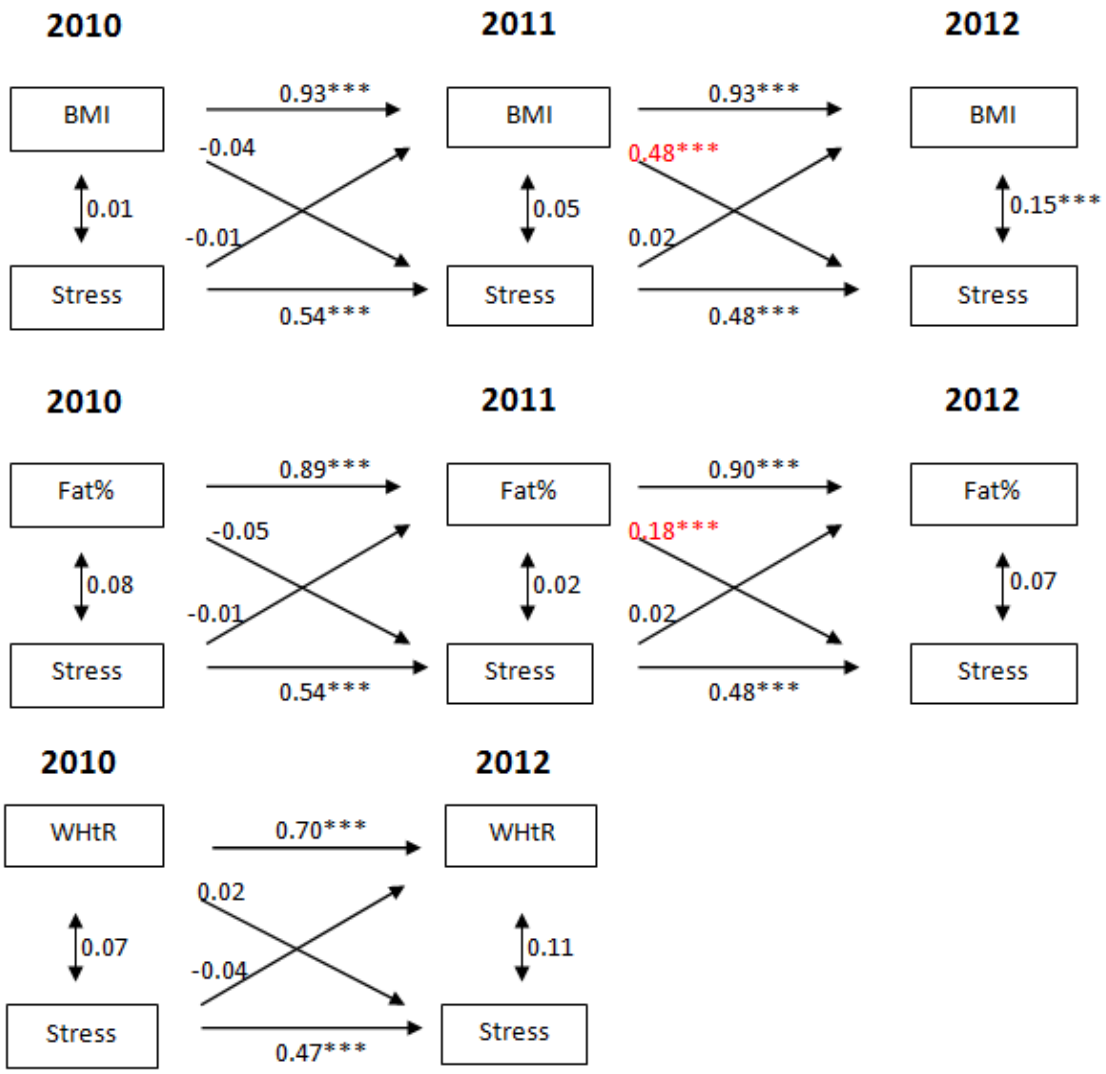


Figure 29: Longitudinal cross-lagged models for composite stress score – adiposity.

*Standardized coefficients are given for forward and reverse associations between adiposity and composite stress score. Fit indices were: CFI=0.983 and RMSEA=0.024 for body mass index; CFI=0.978 and RMSEA=0.026 for fat%; CFI=0.914 and RMSEA=0.045 for waist-to-height ratio. The models are adjusted for age, sex and socio-economic status.*P<0.05, **P<0.01, ***P<0.001*

Table 36: Age differences in longitudinal effects of adiposity (2011) on stress levels (2012)

	Young (6-8y in 2011)		Old (9-11y in 2011)	
	Bèta coefficient	p	Bèta coefficient	p
Total problems				
BMI	-0.070	0.504	0.235	0.036*
Fat%	0.008	0.952	0.307	0.012*
Waist-to-height ratio	0.158	0.172	0.303	0.019*

*The cross-lagged models are adjusted for sex and socio-economic status.*P<0.05, **P<0.01, ***P<0.001*

Moderation by cortisol

Significant stress-cortisol interactions in predicting adiposity (cortisol moderation) are depicted in Figure 30. Only in the case of high AUCg, high AUCi or steep decline, a positive longitudinal relation was shown between reported stress and adiposity.

Moderation by lifestyle

Lifestyle factors could moderate the longitudinal stress on adiposity relation. Figure 31 shows that moderation was significant for screen time, sedentary time, moderate-to-vigorous physical activity and sweet food consumption. It was not significant for the other tested lifestyle parameters (snacks, fatty food consumption, fruit and vegetables, eating behaviour, physical activity and sleep). The moderations show that stress increased adiposity in children with high sweet food consumption and long screen time. On the contrary, low sedentary time or more physical activity could lead to an adiposity lowering effect of stress. When repeating the analyses for the different stress concepts (events, emotions, problems), sweet food consumption was a moderator in the relation emotions-WHtR, events-fat% and emotions-BMI and moderate-to-vigorous activity was a moderator in the problems-BMI relation.

Mediation

Lifestyle factors were no mediators in the stress – adiposity relation since stress was not related to changes in adiposity.

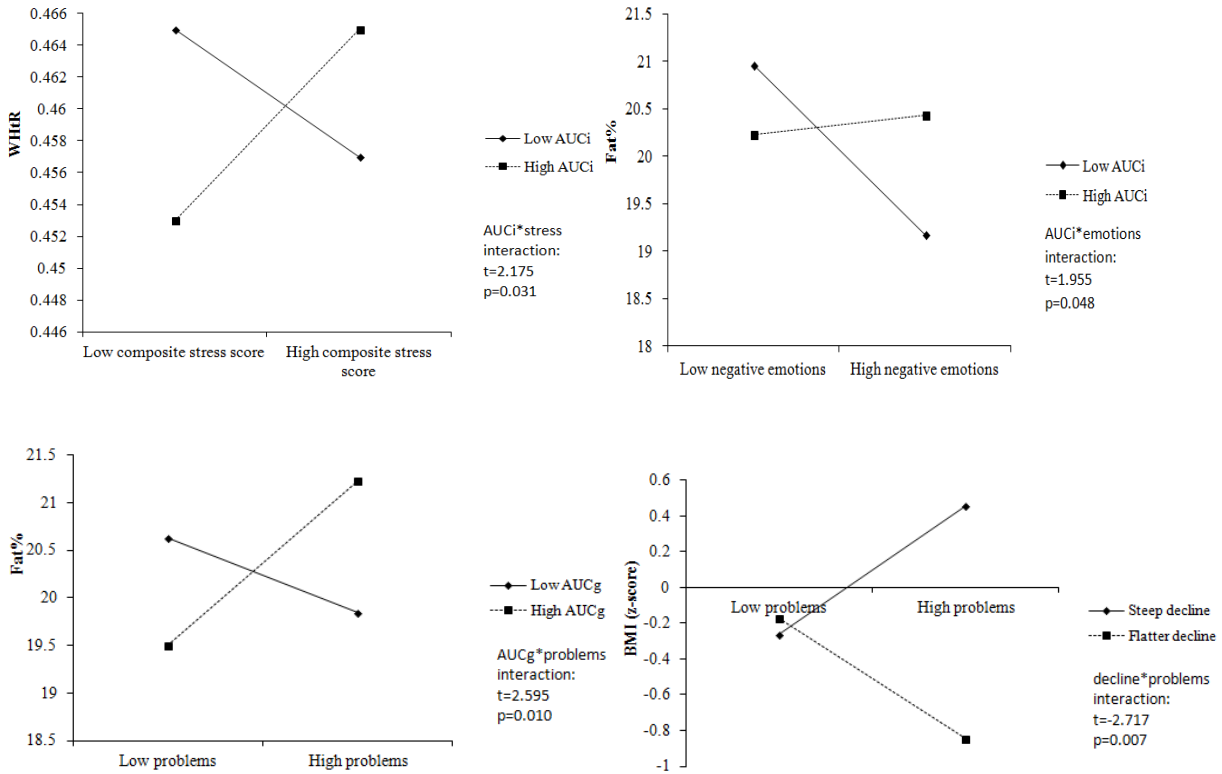


Figure 30: Cortisol as moderator in the stress – adiposity relation

The graphs illustrate the linear regressions in which cortisol was a significant moderator. The stress – adiposity relation is visualized for moderator (cortisol AUCi, AUCg and diurnal decline) and stress levels (composite stress score, negative events, negative emotions or problems) 1 standard deviation above the mean (=high) and 1 standard deviation below the mean (=low). AUCg= area under the curve with respect to the ground; AUCi= area under the curve with respect to increase; BMI= body mass index (z-score); WHtR= waist-to-height ratio

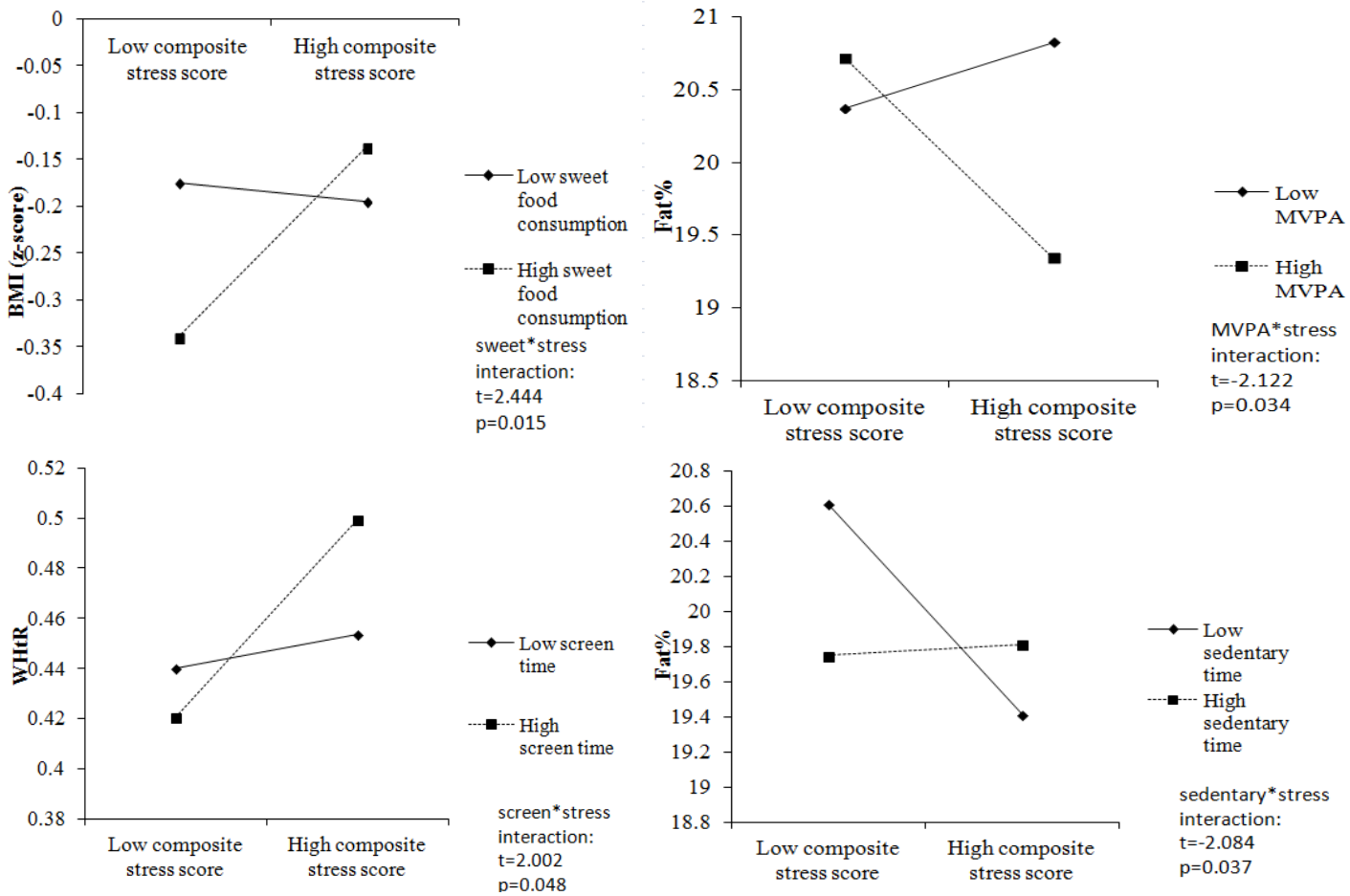


Figure 31: Lifestyle as moderator in the longitudinal stress (composite score) – adiposity relation

Figures illustrate the associations in which lifestyle was a significant moderator as found by interaction in Mplus cross-lagged analyses. The longitudinal relation between the composite stress score and adiposity (BMI, fat% or WHtR) is visualized for moderator and stress levels 1 standard deviation above the mean (=high) or 1 standard deviation below the mean (=low). WHtR= waist-to-height ratio; MVPA= moderate-to-vigorous physical activity.

Discussion

The aim of this study was to examine the cross-lagged, bidirectional relation between stress and adiposity in about 300 primary school children. Moreover, moderation and mediation of cortisol and lifestyle in the effects of stress on adiposity were tested. (1) Evidence was found for a direct relation in the adiposity-stress direction: adiposity was related to higher stress in a longitudinal way (especially negative emotions, but also behavioural problems in the oldest group). (2) No direct effect in the stress-adiposity direction was found. (3) Nevertheless, a stress effect on adiposity was observed when considering cortisol (awakening response and diurnal decline) and lifestyle (sedentary time, screen time, moderate-to-vigorous activity and sweet food consumption) as moderators in this relation. Stress increased adiposity in children with high cortisol patterns, high sweet food consumption or long screen time. On the contrary, especially low sedentary time or high physical activity could lead to an adiposity lowering effect of stress.

Even in this population with low overweight prevalence, body composition had an impact on stress since adiposity induced stronger negative emotions. Several underlying pathways have been suggested in literature. Physiologically, adiposity increases circulating pro-inflammatory cytokines and leptin levels which eventually may stimulate cortisol secretion (Foss and Dyrstad 2011). Psychologically, obese people may face lower self-esteem and negative emotions by external stigma, internal body image dissatisfaction, failing repeated dieting, functional impairment and lower self-rated health (Markowitz, Friedman et al. 2008). Indeed, mainly negative emotions were predicted by adiposity in our sample. In the older age group, also behavioural problems were predicted by adiposity. This was in line with a previous study in 3 to 5 year olds (Griffiths, Dezateux et al. 2011). Since these stress-adiposity relations may be bidirectional, the body composition of people with psychosocial symptoms should be monitored, but – perhaps more important – mood should be monitored in overweight patients too. Consequently, prevention and intervention strategies should target both aspects.

In the opposite cause-effect direction, no significant simple relationship was retrieved. Of course, longitudinal effects of stress on adiposity could be thwarted if adiposity-controlling lifestyle factors are differentially influenced by stress (Dallman, Pecoraro et al. 2003, Buckley and Schatzberg 2005, Akerstedt 2006, Tsatsoulis and Fountoulakis 2006, Adam and Epel 2007, Kappos 2007, Torres and Nowson 2007, Biddle and Asare 2011, Balantekin and

Roemmich 2012, Epel, Tomiyama et al. 2012). Previously, we have shown that stress longitudinally increased physical activity (obesity reducing) and also increased unhealthy diet or eating patterns (obesity promoting) in our child population (Michels, unpublished). Actually, our results revealed individual differences in the stress-adiposity relation depending on lifestyle but also on the physiological stress reactivity (cortisol levels).

The lifestyle factors diet, activity and sleep have been suggested as key parameters in the stress-adiposity relation since they may be influenced by stress and could promote adiposity as well (Pervanidou and Chrousos 2011). Yet, the evidence on intermediate and moderating factors is scarce. More specifically, low physical activity and an unhealthy diet were mentioned as mediators for the effect of mental health on obesity (Gatineau and Dent 2011). Since no direct longitudinal effect of stress on adiposity was seen in our population, mediation could not occur. Nevertheless, some lifestyle parameters were found to moderate the stress-adiposity relation. Diverse stress-adiposity relations may exist because of inter-individual differences in lifestyle behaviour depending on individual characteristics (sex, age, stress reactivity, behavioural preferences and eating behaviour) and/or exposure characteristics (type and severity of stressor) (Becker, Berkley et al. 2008, Macht 2008, Roemmich, Lambiase et al. 2011, Balantekin and Roemmich 2012). Consequently, an unhealthy lifestyle may make children more prone to stress-induced adiposity or, vice versa, a healthy lifestyle may attenuate the pivotal stress effects on adiposity or even lower adiposity. In the current study, the first statement was applicable to high sweet food consumption and screen time; the second statement was applicable to low sedentary time and high physical activity.

An association between high sedentary screen time and poorer mental health in children has been reported in a recent review (Biddle and Asare 2011). Literature also indicates that the stress impact on screen time could depend on usual screen time (Balantekin and Roemmich 2012) and on stress reactivity (Roemmich, Gurgol et al. 2003). Although no moderating effect of sedentarism has been described in literature, physical activity was buffering (i.e. moderating) the effect of stress on adiposity or metabolic syndrome in adolescents (Yin, Davis et al. 2005, Holmes, Eisenmann et al. 2008). Moreover, physical activity has been stated as a buffer for stress effects on overall health (Gerber and Puhse 2009).

The moderating effect of diet is even more theoretically grounded. Stressed people may eat increased amounts of unhealthy food as eating is a way to cope with stress (distraction) (Macht 2008) and stress can influence reward and appetite pathways (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Torres and Nowson 2007, Epel, Tomiyama et al. 2012). Some

specificity in this relation has been suggested with eating being stimulated by mainly moderate (not very intense) emotions and in the presence of emotional or restrained eating (Macht 2008).

Cortisol was a biological moderator in the stress-adiposity relation. This was seen for both the cortisol awakening response (AUCi and AUCg) and the diurnal decline. The composite stress score was only related to the AUCi parameter which reflects the pure increase and as such the sensitivity of the cortisol system. Stress reports are not always associated with chronically elevated cortisol patterns. Variability in cortisol response is partially attributable to the nature of the stressor (type and controllability) and also to the person facing it (emotional response and psychiatric sequelae) (Miller, Chen et al. 2007). The uncovered stress-cortisol interactions in predicting adiposity imply that only children with elevated cortisol levels in response to stress are more susceptible to stress-induced adiposity. This moderating effect of cortisol encourages further research on determinants for chronically elevated cortisol levels (e.g. personality or stressor characteristics) to identify those children that are most vulnerable to stress-induced adiposity. A study in 8-11y old girls also confirmed cortisol as a moderator in the relation between stress events and abdominal fat: a higher number of school-related negative events was related to more abdominal fat for girls with a high cortisol awakening response but no such association was found for girls with a low cortisol awakening response (Donoho, Weigensberg et al. 2011). Another study demonstrated a mediating effect of cortisol reactivity in the depression-adiposity relation in 8-13y old girls (Dockray, Susman et al. 2009).

Some discussion in literature exists on which age group is most vulnerable to stress-induced adiposity. A study in 4065 adolescents reported consistent cross-sectional stress-obesity relations during four measurement waves but no longitudinal relations, suggesting that the relations may already be established during childhood (van Jaarsveld, Fidler et al. 2009). Also in a sample of 2278 children, behavioural problems during childhood predicted adiposity better than those during adolescence (Mamun, O'Callaghan et al. 2009). In contrast, other research hypothesises that adiposity development would be more clearly visible during adolescence and adulthood (Tanofsky-Kraff, Cohen et al. 2006, Moreno, Pigeot et al. 2011). Even though we found no age difference, the effect on adiposity can be expected to be larger when following the children for a longer period of time.

A final frequently discussed issue is the stress effect on fat distribution. Mechanistic studies state that stress might preferentially lead to central adiposity due to the higher density of cortisol receptors in the abdominal region (Bjorntorp 2001). In our study, stress effects were indeed seen on the WHtR, but also the general adiposity parameters BMI and fat% were significantly influenced by stress.

Strengths and limitations

A central asset of this study is the longitudinal design revealing the directionality of the relation, in contrast to the literature that is mainly based on cross-sectional findings. Moreover, this is one of the first studies examining the stress-adiposity relation in children while also considering the moderating role of biological stress measures (i.e. salivary cortisol) and lifestyle factors. Several stress concepts (events, emotions, problems) and lifestyle factors (diet, eating behaviour, reported and measured physical activity, sedentary time and sleep duration) have been measured. For adiposity, overall adiposity measures (BMI and the more advanced fat% determination) as well as a central adiposity measure (waist circumference) have been used.

Nonetheless, our results in Belgian children cannot be generalized to the overall population. First of all, cultural variations have been described in stressors and coping (Chen and Kennedy 2005), which could change the moderating role of lifestyle factors. Even more important, our population showed a low overweight prevalence (8%). Probably stronger associations between stress and adiposity might be encountered in more overweight populations. Another limitation is that not all variables were collected during all three waves: cortisol was only measured at baseline due to high burden and costs, while some other variables were only measured during two waves. A considerable drop-out was encounter during follow-up, although this did not introduce large bias. Finally, longer follow-up studies would be necessary to study the effects of stress on obesity development. Although these observational bidirectional analyses might broaden insight in the directionality of the relation, they are no proof for real causal relations.

Conclusion

The results of this paper demonstrate that stress can increase adiposity when children have high cortisol patterns and/or an unhealthy lifestyle (high sweet food consumption and low

physical activity). Although stress is not always inevitable, the way people cope with stress can be targeted. This emphasizes the value of incorporating education on stress management in obesity prevention programs. Since lifestyle behaviour can also be applied as a coping mechanism and because it moderates the stress-adiposity relation, multi-factorial obesity prevention programs should be created by focusing on stress and concurrently on several lifestyle factors. In targeting lifestyle, children and their parents should realise the effects of stress on their diet and an activity-friendly environment should be created to minimize sedentary time.

Notably, our results also highlighted the unfavourable effect of adiposity on psychological health. This underlines the need for a trans-disciplinary obesity treatment with a special focus on psychological support.

VI.

GENERAL DISCUSSION

1. Summary of results

The **ChiBS study** was designed to investigate the relationship between chronic psychosocial stress and changes in body composition (adiposity) over a two-year follow-up period (2010-2012) in a non-clinical population sample of young children (5-12 years old)

It was hypothesized that chronic stress may promote adiposity **directly through hormonal increase of fat** deposition **and/or indirectly by lifestyle factors** such as the increased consumption of energy dense highly palatable (sugar and fat rich) foods and a deviant eating behaviour, decreased quantity and quality of sleep and a decreased amount of physical activity (with an increase in sedentary screen time). A second, parallel aim was to test the **feasibility and interrelationships of different stress measurements** in children. After all, child- and parent-reported stress data as well as objective stress biomarkers (salivary cortisol and HRV) were used to accurately measure stress. To accurately describe adiposity, a body fat measurement with the advanced ADP technology was used apart from BMI. Also waist circumference was measured as an indicator for the more central fat deposition. The lifestyle factors that were considered in the effects of stress on adiposity were diet, eating behaviour, physical activity, sedentary behaviour and sleep (quantity and quality).

The **cross-sectional findings** are summarized in Figure 32 and the **longitudinal relations** in Figure 33. First, percentile values for salivary cortisol and HRV were determined. Next, the following relationships were tested cross-sectionally: the comparison of different **stress measures** (cortisol vs HRV vs questionnaires), the comparison of different **body composition indicators**, the sleep-adiposity relation, the **stress-lifestyle** relation and the **stress-adiposity** relation. Longitudinally, the stress-lifestyle and stress-adiposity relation were retested.

Overall, the results showed that childhood stress is a key determinant for endocrinological changes and longitudinal lifestyle changes (mainly unhealthy diet and eating behaviour) that further might stimulate adiposity. Although no direct longitudinal effect of stress on adiposity was detected, lifestyle and cortisol moderated the stress effects on adiposity. Consequently, both the endocrinological pathway and the lifestyle pathway in the stress effects on adiposity may be important. Moreover, the lifestyle pathways will probably play a bigger role when children grow older since adolescents will have more independence over personal choices including diet and physical activity. Next, also an inverse relation was found: adiposity and maladaptive eating behaviours increased the stress level in these children.

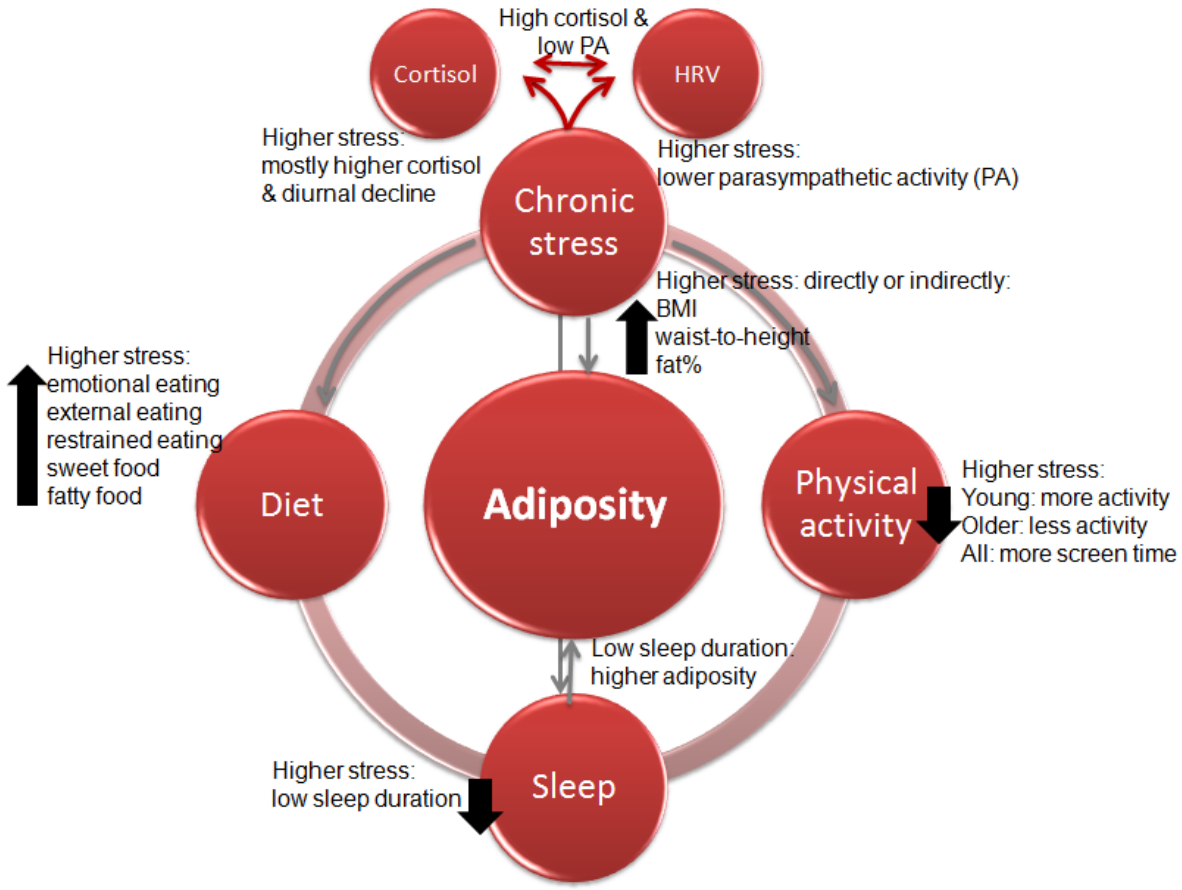


Figure 32: Cross-sectional findings in the ChiBS study.
ADP= air displacement plethysmography, HRV= heart rate variability, PA= parasympathetic activity

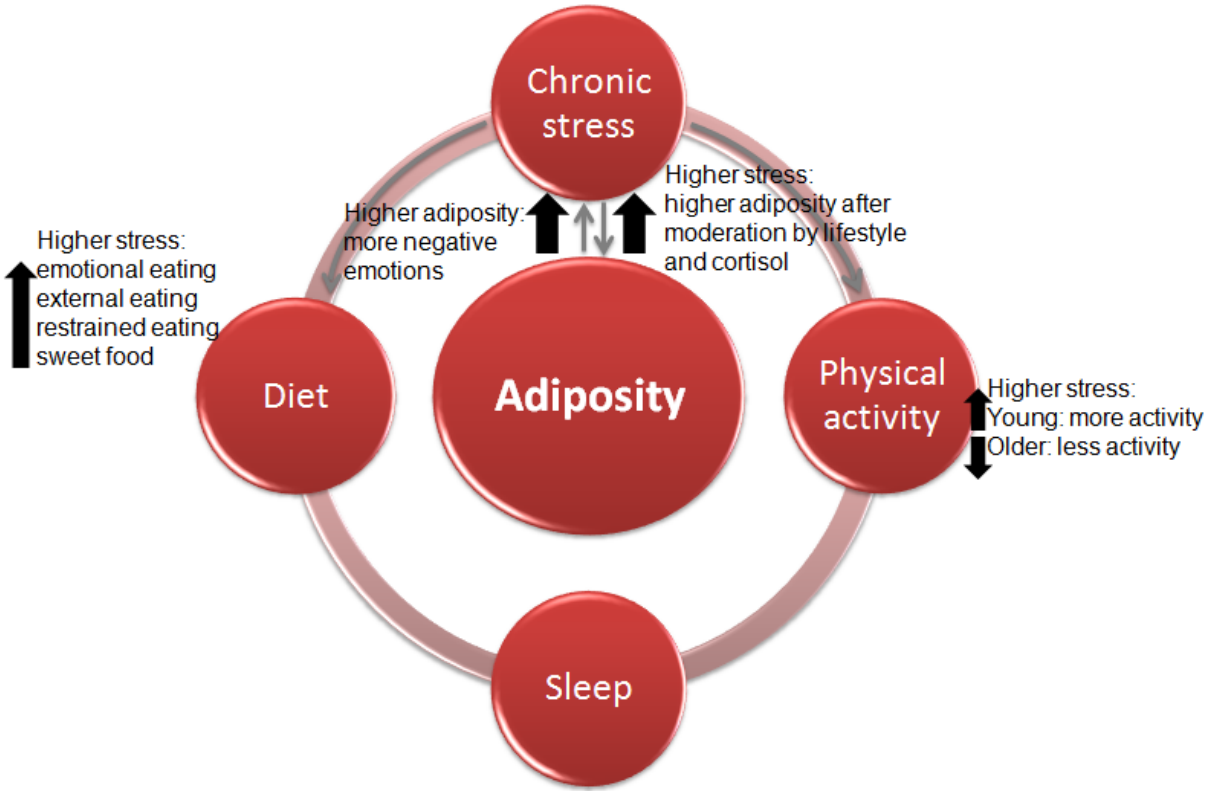


Figure 33: Longitudinal findings in the ChiBS study.

1.1. Measurement of stress in children

(Based on chapter III.1, III.2, III.3 and III.4)

In analysing stress, the two most important stress-pathways were covered in this study, using cortisol measurements representing the HPA stress system and HRV representing the ANS. Determinants and intercorrelations of the stress measurements were evaluated. Moreover, questionnaires were used to collect psychometric data on stress exposure in the participating children.

Children's salivary cortisol values, especially the absolute increase in the morning, had a rather poor intra- and inter-individual stability over time, emphasizing the **need for multiple day sampling**. In only 52.5% of the children, a CAR was observed. The parent-induced time lag and also the unintended time lag caused by transient awakening are the most plausible factors for CAR absence, although the possibility of CAR absence as a general characteristic in young children cannot be excluded. Main contributors to the variance in cortisol levels were age, time compliance and awakening time. This indicates the importance of using an elaborated manual and checklist to promote compliance and **exclude large deviations from the instructed time** schedule, especially in the morning. Furthermore, **corrections for age and awakening time** are necessary. After exclusion of time noncompliers and corticosteroid users, age-specific percentile values were computed as guide for endocrinologists.

Significant determinants of HRV were **age and sex**: lower HRV in girls and wave-like parasympathetic increases and sympathetic decreases with increasing age. Consequently, sex- and age-specific reference values were produced. Adiposity was no determinant of HRV but **fat-free mass, physical activity and in particular physical fitness** had a favourable association with higher parasympathetic activity.

The associations of cortisol and HRV with stress questionnaire data are shown in Figure 34. The relation between questionnaire data and cortisol levels predominantly supported a **cortisol stimulating effect of stressors** in children, although lower cortisol values were detected for peer problems in girls. The relation between questionnaire data and HRV levels predominantly supported a **decrease in parasympathetic activity** in children exposed to stress.

The two markers **HRV and cortisol showed mutual moderate associations**. Consequently, both cortisol and HRV can be used as stress marker in children. Measuring both pathways is

still recommended as they might be stimulated differently depending on the stressor or stress outcome as shown in Figure 34.

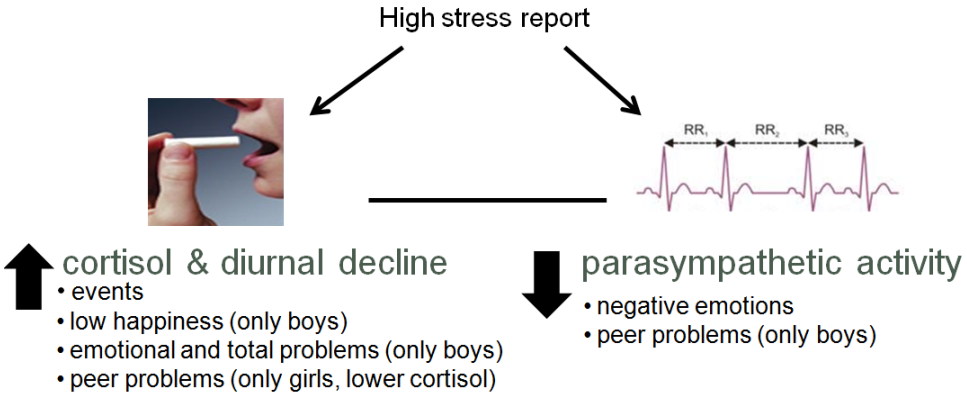


Figure 34: Relation of stress questionnaire data with cortisol and heart rate variability.

1.2. Measurement of adiposity

(Based on chapter III.5)

In this study, an advanced method of body composition determination was used: the BOD POD® device based on ADP technology. Large-scale epidemiological studies are often restricted to routine measurements because of the logistic and budgetary constraints of the ADP technology. Therefore, the relative validity of routine measurements was tested by analysing the association of fat% assessed by ADP with 1) anthropometric measurements/indices (based on weight, height, skinfold thickness, body circumferences and bioelectrical impedance) and 2) fat% assessed with equations based on skinfold thickness and bioelectrical impedance.

The sum of skinfold thicknesses showed the highest correlation with ADP fat%, followed by triceps skinfold, arm fat area and subscapular skinfold. Concerning the equations to assess fat% based on anthropometry, the Tanita® equation scored best of all impedance equations and the Deurenberg equation performed best of all skinfold equations. Anyhow, none of the used equations were interchangeable with fat% assessed with ADP. The agreement between ADP and equations also showed age and sex effects and an increasing underestimation with increasing fat%. In conclusion, the **sum of triceps and subscapular skinfold thickness is recommended as marker of fat% in the absence of specialized technologies.** Nevertheless, the higher cost and logistic efforts of an immobile but more advanced device like the BOD POD remains justified. After all, the error by using skinfolds increases with increasing fat%.

1.3. Stress-lifestyle

(Based on chapter IV.1, IV.2, IV.3 and IV.4)

Two main pathways were hypothesized in the effects of stress on adiposity. Firstly, **direct metabolic changes** (such as increased visceral fat disposition and appetite stimulation) may be caused by a dysregulation of the stress system and the production of stress hormones (mainly cortisol). Secondly, stress may indirectly facilitate obesity through **behavioural pathways** such as maladaptive coping behaviours leading to an adiposity stimulating lifestyle: emotional eating of 'comfort' food, a disordered sleep and a lack of exercise with an increase in screen time.

Diet is the most thoroughly examined lifestyle factor in the stress effects on adiposity.

Salivary cortisol patterns that might reveal higher stress levels (hypercortisolism reflected by both overall higher levels, steeper diurnal slope and more prominent CAR) were associated with an unhealthier dietary pattern: higher fatty food and snack consumption frequency, but especially higher sweet food consumption frequency. No associations were found with fruit & vegetables consumption frequency. These results suggest the **cortisol-induced comfort food preference** that might explain the relation between stress and an unhealthy diet.

Cross-sectionally, stress questionnaire data was linked to more sweet food, fatty food and fruit & vegetables, but only sweet food and fatty food consumption were higher when the total stress score was higher. **Longitudinally, only sweet food consumption frequency** was increased by stress but this was restricted to the **oldest group of children**.

Apart from dietary intake, also eating behaviour was connected with stress questionnaire data. Cross-sectionally and longitudinally, all three eating behaviours (**emotional, external and restrained eating**) were associated with a higher stress score. The relations were depending on the used stress construct and children's age and sex. Although both emotional eating and dietary patterns were associated with stress, no association was found between emotional eating and dietary patterns. Consequently, emotional eating behaviour was no mediator in the stress – diet association.

A second examined lifestyle parameter was activity. **Cross-sectionally,** lower **objectively measured physical activity** (but not reported activity) was associated with the total stress score and more specifically with negative emotions. Age moderated the relation such that

higher physical activity was found in the younger stressed children and lower physical activity in the older stressed children. **Longitudinally, both objective and reported physical activity** were augmented by higher total stress, more negative emotions and more negative events. Again opposite effects were seen depending on the age: **augmented physical activity in the youngest age** group and **less activity in the oldest group experiencing more stress**. Taken together, the stress effects on physical activity are largely depending on children's age. Objectively measured sedentary behaviour and reported screen time revealed no longitudinal relations with stress.

A last examined lifestyle parameter was sleep. **Only cross-sectionally, lower sleep duration** was found in children with high stress reports (mainly negative emotions). **No longitudinal relations** were detected. Apart from sleep duration, also sleep quality was measured at baseline. Interestingly, we could not find a relation between sleep quality and adiposity parameters. In addition, **low sleep quality was only associated with conduct problems in girls** (unpublished results: lower sleep efficiency β=-0.234 p=0.013; more awakenings β=0.216 p=0.025).

The observed stress-lifestyle relations were **mainly unidirectional: stress influencing lifestyle**. Only the maladaptive eating behaviours could deteriorate emotional status (anxiety). This stress-emotional eating bidirectional relation might possibly lead to a vicious circle.

1.4. Stress-adiposity

(Based on chapter V.1 and V.2)

Cross-sectionally, childhood stress was positively related to **both overall and central (waist-to-height ratio) adiposity** measures. Negative life events were positively and happiness was negatively associated with adiposity. Peer problems were positively associated with adiposity in girls only.

Longitudinally, the relations were tested in both directions (see Figure 35). **Stress did not directly affect adiposity** but **adiposity had a direct influence on stress**: a higher BMI or fat% resulted in stronger negative emotions and in the oldest group also in more behavioural problems. In studying the effects of stress on adiposity, **lifestyle factors and cortisol were significant moderators** (but no mediators). As a result, significant stress on adiposity relations were found in the group with high cortisol and unhealthy lifestyle for both overall

and central adiposity. Screen time, physical activity and sweet food were the longitudinal moderating lifestyle factors in these stress on adiposity relations.

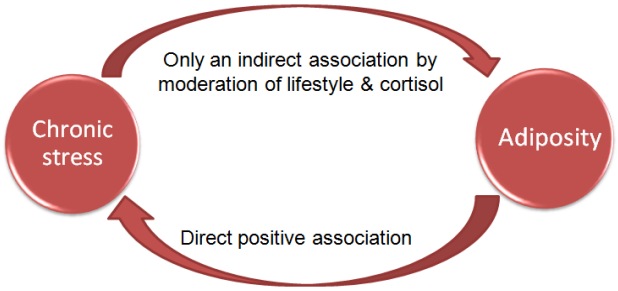


Figure 35: Simplified summary of the bidirectional stress-adiposity relations

2. Discussion of results

The ChiBS cohort study provides valuable data and **new insights** concerning the influence of chronic psychosocial stress on adiposity changes, and its interaction with food intake regulation, physical activity/sedentary behaviour and sleep in young children. In addition, this study allowed an in-depth investigation of the validity of the different methods that were used to assess stress levels in children. These longitudinal results give more insight in potential causal relations and **underlying mechanisms that can be used for prevention**, although experimental studies are still necessary to prove these mechanisms.

Overall, the study emphasizes **the importance of childhood stress** since stress could influence endocrinology, lifestyle behaviour and consequently health in children. Childhood is a period of incessant physical and brain development. Adult psychopathology (Fryers and Brugha 2013), lifestyle (Thorleifsdottir, Bjornsson et al. 2002, Mikkila, Rasanen et al. 2005, Ashcroft, Semmler et al. 2008, Telama 2009) and adiposity (Singh, Mulder et al. 2008) can all find their foundations in childhood. Also in children, chronic alterations in stress activity may have permanent effects on endocrine and metabolic systems (Teicher, Andersen et al. 2002, McEwen 2008).

2.1. Methodological results

During this PhD research, it was found that (1) HRV measurements should be corrected for age, sex and physical activity/fitness when being used as indicator for stress and that (2)

cortisol measurements should be corrected for age and awakening time after excluding time noncompliers. Moreover, percentile values for salivary cortisol and HRV were presented. The methodological analysis on body composition indicators suggested the use of the sum of triceps and subscapular skinfold in the absence of less sophisticated technologies like ADP, although it is still not as accurate as ADP. These methodological results will not be further discussed since they served mainly as methodological background for studying the effects of children's stress on endocrinology, lifestyle and adiposity.

2.2. Cortisol and HRV as stress marker

Children's stress questionnaire data was associated with the biomarkers of the two important stress systems: cortisol and HRV.

The reported stress-cortisol associations in literature are quite complex, giving conflicting results. First of all, the study of stress-cortisol relations could be thwarted by genetic differences since e.g. cortisol morning values are up to 30% genetically defined (Van Hulle, Shirtcliff et al. 2012). More importantly, a **hyper-/hypocortisolism hypothesis** was published suggesting that recent exposure to a stressor may initially elevate cortisol levels, while the HPA axis may develop a counter-regulatory response of cortisol lowering after extended exposure to stress (Heim, Ehlert et al. 2000). The question remains whether this statement is also applicable in children. Although some studies found this hypocortisolism pattern in children (Gunnar and Vazquez 2001, Bruce, Fisher et al. 2009), hypocortisolism may be less frequent in children as some time is needed before the counter-regulatory response is induced. A recent study showed that adversities during childhood were associated with high cortisol levels, while adversities during adolescence were associated with low cortisol levels (Bosch, Riese et al. 2012). The relation between questionnaire data and cortisol levels in our study indeed predominantly supported a **cortisol stimulating effect of stressors** in children. This could also be a result of our high SES population (with lower stress exposure). After all, hypocortisolism is mainly seen when confronted with severe trauma (Miller, Chen et al. 2007) or an accumulation of several stressors (Gustafsson, Anckarsater et al. 2010).

In contrast with this hypercortisolism, **lower cortisol values were detected for peer problems in girls.** As far as we know, no other studies have demonstrated this sex difference in our age group. Previously, lower cortisol was observed to be related to lower peer status at school in 15-year olds (West, Sweeting et al. 2010), although a study in preschoolers showed

highest median cortisol in the least liked/most disliked children (Gunnar, Sebanc et al. 2003). Sex differences in children's peer context has been reviewed with girls being more sensitive to the status of their friendships, more exposed to a wide variety of peer stressors and receiving higher levels of emotional provisions in their friendships (Rose and Rudolph 2006). As such, peer problems may create a strong chronic stress situation in children leading to hypocortisolism.

The use of HRV as a stress marker is common in adults, but the application in children is still scarce. Literature is especially abundant on the HRV relationship with anxiety, e.g. anxiety in adults and children was related to low parasympathetic and high sympathetic activity (Friedman 2007). The relation between questionnaire data and HRV levels in our study predominantly supported a **decrease in parasympathetic activity** with more negative emotions. Indeed, a vagal circuit in the physiological regulation of emotions has previously been outlined (Porges, Doussard-Roosevelt et al. 1994). Additionally, also a higher sympathetic over parasympathetic dominance was found for anxiety and anger. These findings suggest that HRV can also be used as a stress marker in children. Given that a reduction of HRV (i.e. reduced parasympathetic with or without increased sympathetic activity) is a pathway of increased morbidity and mortality (Thayer, Yamamoto et al. 2010), HRV may be a potential pathway linking stress to ill health (Thayer and Brosschot 2005).

Up to now, only few studies examined the **HRV-cortisol** relation in children or adults and those who did handled particularly about the CAR (Eller 2007, Stalder, Evans et al. 2011). Nevertheless, some studies did not find significant association between cortisol and HRV (Gunnar, Porter et al. 1995, Johnson, Hansen et al. 2002). Despite the shown associations between HRV and cortisol, their reaction on stress may differ. For instance, a dissociation has been reported in the reactivity of both neural systems to repeated stressors with a lower cortisol stress response after several repeated exposures (=habituation), while the HRV reaction remained high (Schommer, Hellhammer et al. 2003). In our study, moderate associations were found between HRV and cortisol. A high cortisol level and steep diurnal decline were associated with low parasympathetic activity, while a high CAR was mainly associated with a sympathetic over parasympathetic dominance.

Interestingly, different associations were seen: HRV was mainly associated with negative emotions, while cortisol was mainly associated with negative events and lower happiness. This different association may indicate differences in the activation of the systems underlying both biomarkers. Although both HRV and cortisol are valid stress markers, **measuring both pathways is recommended** as they might be stimulated differently depending on the stressor or stress outcome.

2.3. Stress and lifestyle

Dietary pattern, psychological eating behaviour, physical activity, sedentary behaviour and sleep were studied as lifestyle factors that might potentially be influenced by stress.

During stressful periods, there is a change in what type of food is eaten, independent of the total intake (Gibson 2006). The type of food ingested shifts toward the more palatable i.e. the comfort food. This comfort food can be used as a way to distract from stress (Macht 2008) and is enhanced due to the stimulated reward pathways by stress (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Torres and Nowson 2007, Epel, Tomiyama et al. 2012). In preadolescent children research is scarce. To our knowledge, only one study in preadolescents demonstrated more snacking with more perceived stress (Jenkins, Rew et al. 2005) and one study showed more snacking in response to laboratory stressors (Roemmich, Wright et al. 2002). In our study, stressed children had an unhealthier diet, but in the long term this was only reflected in higher **sweet food consumption**. Consequently, mainly the sweet taste of unhealthy items (not the healthy sweet fruits) might be defined as 'comfort food' in our paediatric population. The higher independence older children have over their own food choice compared to the higher parental control in younger children may be a possible explanation for the stress-food relations being only significant in the oldest group.
An underlying mechanism in the stress-diet relation could be the increased cortisol levels that stimulate reward and appetite pathways (Dallman, Pecoraro et al. 2003, Adam and Epel 2007, Torres and Nowson 2007, Epel, Tomiyama et al. 2012). Studies examining the cortisol-diet relation are mainly based on animal studies (Dallman, Pecoraro et al. 2003) or human laboratory studies (Epel, Lapidus et al. 2001, George, Khan et al. 2010). Population studies have largely used subjective stress reports instead of cortisol to study the effect on diet, although a biological marker would increase physiological understanding (Torres and Nowson 2007). Salivary cortisol patterns that might reveal higher stress levels were

associated with an unhealthier dietary pattern in our study: higher fatty food and snack consumption frequency, but especially higher sweet food consumption. This is in agreement with the longitudinal stress-induced intake relation of sweet food in our population. These results suggest the **cortisol-induced comfort food preference** that might explain the relation between stress and an unhealthy diet.

Apart from diet, also eating behaviours were examined since they eventually can lead to increased or unhealthier food consumption. Stress increased **emotional, external and restrained eating** in an age, sex and stress construct dependent manner. In the scarce literature on children and adolescents, negative emotions and problems have been associated mainly with emotional eating (Braet and Van Strien 1997, Goossens, Braet et al. 2009, Nguyen-Rodriguez, McClain et al. 2010). Indeed, people with an emotional eating behaviour have learned to label the negative feelings of stress as 'hunger' (Bruch 1964) and will think about food as an escape from stress (Dallman, Pecoraro et al. 2003, Adam and Epel 2007). In contrast, less evidence for stress induced changes in external and restrained eating is available. Emotions have been hypothesized to impair cognitive control to external stimuli such as food (Macht 2008), which can possibly explain the link we found with negative events and problems.

Remarkably, the maladaptive eating behaviours could also increase negative emotions like anxiety in our population. This **stress-emotional eating** bidirectional relation might possibly lead to a vicious circle. Consequently, children and their parents should be made aware that stress can influence their diet. Moreover, problem-solving coping skills should be highlighted as an alternative for coping by food.

Increased screen time was only cross-sectionally related to stress. Longitudinally, stress **increased** physical activity in our childhood sample. Of course, physical activity can be used as way to cope with stress since walking/running was reported by a group of children as a frequently used and efficient coping strategy (Chen and Kennedy 2005). Also in a laboratory experiment, physical activity increased but only in those that have a high usual level of physical activity (Balantekin and Roemmich 2012). Literature has also shown that physical activity can serve as a buffer for stress effects on adiposity (Yin, Davis et al. 2005), the metabolic syndrome (Holmes, Eisenmann et al. 2008) or even overall health (Gerber and Puhse 2009). Consequently, stimulating activity as reaction to stress can be considered as an

important health advice because spending time for exercising might be considered as a waste of time in such stress periods.

When stratifying our analyses by age, the positive relation was only significant in the young group and even lower physical activity was found in the older group when confronted with negative emotions. This suggests that stress coping strategies are **age-dependent**. Most probably, this positive finding will disappear when kids grow up into adolescents that have more time constraints for doing physical activity due to other priorities like schoolwork.

Since sleep is the metabolic antagonist of stress (Buckley and Schatzberg 2005, Akerstedt 2006), bidirectional relations have been reported although most evidence exists for an effect of sleep on stress (Gregory and Sadeh 2012). In our study, stress was associated with **shorter sleep duration cross-sectionally** but not longitudinally. Also sleep quality might be important because it influences the actual sleep duration. A review reported stress-induced awakenings and a shift in sleep stages e.g. less slow wave sleep (Kim and Dimsdale 2007). We were able to study the cross-sectional relations of stress with sleep quality but only found one relation: conduct problems were associated with lower sleep quality in girls. Although sleep was the lifestyle factor with the least evidence in our stress analyses, future studies should consider sleep quality in longitudinal perspectives to further unravel this aspect.

2.4. Stress and adiposity

Some childhood stress aspects were positively related to **concurrent overall and central adiposity** measures but no association was observed with salivary cortisol. Although our longitudinal models did **not show a direct longitudinal effect of stress on adiposity**, some **effects appeared after moderation**. Cortisol was a first moderator. Stress reports do not always associate with chronically elevated cortisol patterns. The interaction shows that only children that have this chronically elevated cortisol are more susceptible to stress-induced adiposity. Lifestyle also moderated the stress-adiposity relation. Consequently, an unhealthy lifestyle may make children more prone to stress-induced adiposity changes or, vice versa, a healthy lifestyle may attenuate the effects of stress on adiposity. An underlying reason is that not everybody is reacting to stress in the same way. These individual differences will determine the child's adiposity risk. Although stress is not always inevitable, the way people cope with stress can be targeted. This emphasizes the value of incorporating education on

stress management in obesity prevention programs. Since lifestyle behaviour can also be used as a coping mechanism and as it moderated the stress-adiposity relation, multi-factorial obesity prevention programs should be started by concurrently focusing on stress and several lifestyle factors. In targeting lifestyle, children and their parents should be made aware that stress can influence their diet and an activity-friendly environment should be created.

Longitudinally, significances were also found in the other cause-effect direction. Even in this population with low overweight prevalence, **body composition had an influence on stress** since adiposity leads to stronger negative emotions. Indeed, bidirectional mechanisms in stress-adiposity have been stipulated. Psychologically, obese people may face lower self-esteem and negative emotions by external stigma, internal body image dissatisfaction, failing repeated dieting, functional impairment and lower self-rated health (Markowitz, Friedman et al. 2008). This is in line with the diathesis-stress hypothesis that predisposition factors (diathesis) can interact with the environmental factors to result in a disorder (Hankin and Abela 2005). After all, being overweight in a society that promotes the thin ideal might induce low self-esteem and this scar or predisposition makes this child more vulnerable when confronted with new stressors like bullying. Physiologically, one of the hypotheses is that adiposity increases circulating pro-inflammatory cytokines and leptin levels which eventually may stimulate the HPA axis (Foss and Dyrstad 2011). Since these stress-adiposity relations might be bidirectional, the weight and/or body fat of people with psychosocial symptoms should be monitored, while mood should be monitored in overweight patients. Very recently, a broader initiative focusing on the challenges and potentials in transdisciplinary obesity research was launched (Holm, Børker Nielsen et al. 2013).

2.5. Specificity in the stress-lifestyle and stress-adiposity associations

The discussed relations in stress-lifestyle and stress-adiposity depend on sex, age, the stress construct, the lifestyle factor and the indicator of adiposity (see Figure 36). This indicates that not everybody reacts to stress in the same way.

2.5.1. Stress construct differences

All stress constructs showed one or more associations with lifestyle or adiposity. Concerning the effects on lifestyle, mainly negative events and negative emotions were important. Concerning the indirect effects on adiposity, mainly problems and negative emotions appeared significant. In the effects of adiposity on stress, negative emotions and in the oldest children also problems were relevant. Since all stress constructs showed some association but

sometimes differentially, studies should consider the different stress constructs. Apart from different underlying mechanisms, also the use of different reporters could underlie this finding. A multi-informant design with reports of both children and parents avoids informant-based biases (van Dulmen and Egeland 2011).

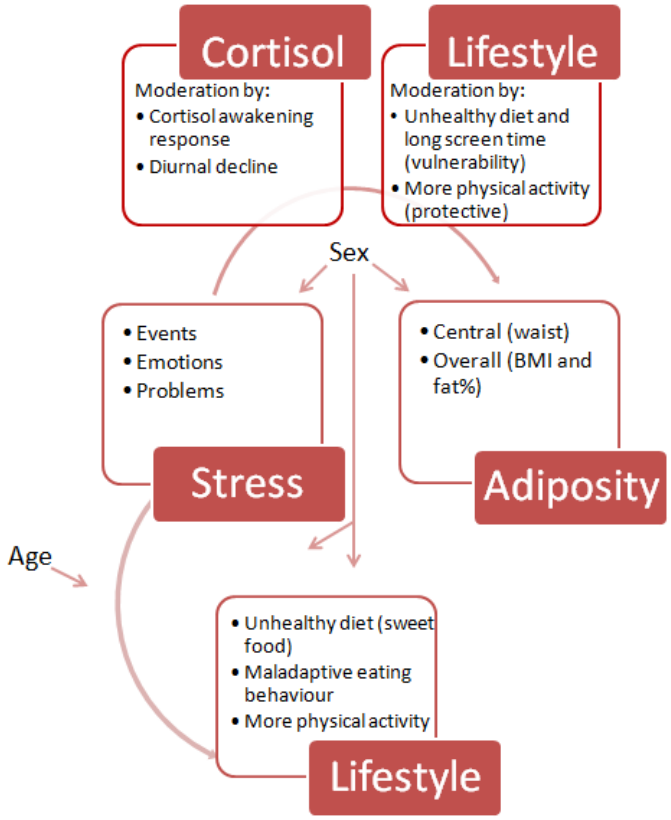


Figure 36: Specificity in the stress-lifestyle-adiposity relation.

2.5.2. Lifestyle differences

Stress had longitudinal unfavourable effects chiefly on **sweet food consumption** and **maladaptive eating behaviours**. The main moderating lifestyle parameter in the stress-adiposity relation was sweet food consumption: a positive stress-adiposity relation was only seen in children with high sweet food consumption. Consequently, sweet food consumption is a vulnerability factor in stress-induced adiposity increase. The same was seen for long screen time. On the other hand, measured physical activity was also a moderator but in a protective way: children being highly physical active (the healthy situation) showed a negative relation between stress and adiposity. Sleep was the lifestyle factor that showed least evidence for a role in stress-adiposity as there was only a cross-sectional relation with stress.

2.5.3. Adiposity differences

Recent meta-analyses and reviews (Luppino, de Wit et al. 2010, Gundersen, Mahatmya et al. 2011, Incledon, Wake et al. 2011, Wardle, Chida et al. 2011) found stress-adiposity relations for **both overall and central** adiposity. Mechanistic studies however stated that stress might preferentially lead to central adiposity due to the higher density of cortisol receptors in the abdominal region (Bjorntorp 2001). When considering both direct and indirect pathways, our results exhibit stress effects on overall (BMI, fat%) and central (waist) adiposity parameters. This again favours the hypothesis that not only cortisol-induced fat increases (with higher specificity for the abdominal region) but also lifestyle-induced fat increases (with no specificity) can explain a stress-adiposity relation.

2.5.4. Age differences

A natural process of children's transition to adolescence is the decreasing parental control. From the age of 10 on, decreasing daily interactions with their family have been found (Larson, Richards et al. 1996). Children's diet and emotional eating behaviour has previously been correlated with that of their parents (Longbottom, Wrieden et al. 2002, Brown and Ogden 2004, van Strien and Bazelier 2007). Nevertheless, a recent meta-analysis/review has shown that the parent-child relation should not be overstated and that there is a trend of diminishing influence the last decade by changing society (i.e. increasing importance of other influencing players like school and peers) (Wang, Beydoun et al. 2011). Moreover, the intake of sweet foods in 12-year olds was influenced by their emotional eating behaviour above the parental influence. In this context, age could be an important factor as in 7-9y old children the perceived parental eating restriction could prevent the child's emotional eating more than in the older group (van Strien and Bazelier 2007). The parental influence thus seems to be higher in young children. Also decreased parental influence with age has been reported for sleep duration (Mindell and Owens 2003) and for physical and sedentary behaviour (Smith, Grunseit et al. 2010).

This parental control in young children can hamper studies on children's lifestyle changes e.g. in the effects of stress as we have studied here. Indeed, age differences were found in the effects of stress on lifestyle. Sweet food consumption was only increased by stress in the oldest group. For physical activity, even an opposite effect of negative emotions on objective activity existed: increased activity in the youngest group, but decreased activity in the oldest group. As children grow older, they have **more independence** over food choices and **more**

time constraints for doing sports. To prevent overweight, it therefore becomes increasingly important to make the environment (e.g. at home, at school etc.) an 'activity encouraging, healthy food zone' that minimizes opportunities for stress-induced eating but maximizes opportunities for healthy eating and physical activity.

2.5.5. Sex differences

Table 37 summarizes the sex differences that were found **in the studied parameters and relations**. Although some relations were not sex-dependent, most relations disclosed sex differences depending on the used stress and lifestyle factor.

Sex differences have been suggested **in literature** for several aspects in the stress-lifestyle-adiposity relation.

(1) *Stress endocrinology*: Women generally show an increased responsiveness to stress due to effects of sex hormones on glucocorticoid receptors, the brain CRH system, pituitary responsiveness to CRH, adrenal responsiveness to ACTH and cortisol feedback inhibition (Becker, Berkley et al. 2008). Sex differences in heart rate and HRV are well-described in adults (Umetani, Singer et al. 1998) with lower HRV values in women. In children, data on HRV sex differences is rather scarce: no sex difference (Goto, Nagashima et al. 1997, Fukuba, Sato et al. 2009), an overall sex difference (Faulkner, Hathaway et al. 2003) or an age- and measure- dependent sex difference (Silvetti, Drago et al. 2001, Galeev, Igisheva et al. 2002) has been observed.

(2) *Psychopathology:* Sex differences have been shown in the prevalence, developmental pathways during childhood and manifestation of psychopathology (Crick and Zahn-Waxler 2003) and in handling stressful situations in children (Hampel and Petermann 2005). Sex differences in children's peer context have also been reviewed with girls being more sensitive to the friendship status, being more exposed to a wide variety of peer stressors and receiving higher levels of emotional provisions in their friendships (Rose and Rudolph 2006).

Table 37: Sex differences in the studied parameters and relations.

Stress questionnaires	Girls : more anxiety, sadness and prosocial behaviour
	Boys : more conduct problems
Salivary cortisol	No sex differences
HRV	Girls : lower HRV
Cortisol - questionnaire	Both sexes : life event increase correlated with higher cortisol
	Boys : happiness increase correlated with lower cortisol
	Boys : increase in emotional problems correlated with steeper diurnal slope
	Girls : increase in peer problems correlated with lower cortisol
HRV - questionnaire	Both sexes : anger was associated with lower parasympathetic activity and higher sympathetic over parasympathetic dominance
	Girls : anxiety and sadness were associated with lower parasympathetic activity
	Boys : peer problems were associated with lower parasympathetic activity
HRV - cortisol	No sex differences
BOD POD - anthropometry	Girls : better association
Sleep - adiposity	No sex differences
Cortisol – diet	No sex differences
Stress – lifestyle longitudinal	Both sexes : stress increases physical activity and sweet food consumption
	Girls : stress increases emotional eating and external eating
	Boys : stress increases restrained eating
Stress - adiposity cross-sectional	Both sexes : more events and low happiness are associated with increased adiposity
	Girls : adiposity is positively associated with peer problems
Stress - adiposity longitudinal	No sex differences

HRV= heart rate variability

(3) *Lifestyle*: Although they have a lower overall dietary intake, women tend to prefer more the sweet/fatty food items (Wansink, Cheney et al. 2003). Moreover, women experience enhanced rewarding and enhanced appetitive emotional learning resulting in higher levels of craving and eating disorders (Becker, Berkley et al. 2008). This is probably due to greater oxytocin projection to the nucleus accumbens in women. Women also tend to have more potent satiation reactions to leptin and serotonin while lower satiety reactions to insulin and cholecystokinin (Becker, Berkley et al. 2008). Concerning physical activity, girls are less active than boys (Verloigne, Van Lippevelde et al. 2012). Concerning sleep, a higher susceptibility to sleep disturbances with more detrimental outcomes has been observed in women (Mong, Baker et al. 2011).

(4) *Energy metabolism:* Females have lower resting energy expenditure and lower basal fat oxidation, leading to more energy preservation (Becker, Berkley et al. 2008).

(5) *Adiposity:* In children and adolescents, obesity tend to be higher in boys (Moreno, Pigeot et al. 2011). In adults, women are more likely to develop obesity (Lovejoy, Sainsbury et al. 2009). From puberty on, the sexes also differ in fat partitioning with more gluteo-femoral fat deposition (the hips and thigh) in girls (Staiano and Katzmarzyk 2012).

(6) *The relation stress and adiposity:* Since sex differences have been reported in all the underlying aspects, also the relation between stress and adiposity can be sex-dependent. Anyhow, the literature is inconsistent: most studies report stronger correlations in women and girls (Atlantis and Baker 2008, Gatineau and Dent 2011) especially when focusing on the effect of adiposity on stress, but sometimes higher correlations in men have been reported (Wardle, Chida et al. 2011).

3. Methodological issues and limitations

3.1. Study design

The baseline ChiBS study was **embedded in the European on-going IDEFICS study**. This created the advantage of using an on-going cohort of non-clinical children for whom a **lot of other relevant epidemiological information** was collected. On the other hand, it **restricted the time** scheme for collection and the amount of extra measurements. Moreover, embedding ChiBS within IDEFICS, which is already burdening children and parents, may have decreased participation to the additional ChiBS project.

To increase willingness for participation, we decided to split the informed consent such that the participants could refuse **participation to single examination modules**. Nevertheless, this has led to different participation rates for each examination module and a low number of children participating to the whole ChiBS examination battery. This implies studying sub-groups of ChiBS participants for investigating separate research questions and a rather limited number of children in whom the whole ChiBS hypothesis is examined. Nonetheless, we succeeded in including **326 children that participated in all 3 measuring waves.**

Although the follow-up did not introduce a large bias (no differences in stress and adiposity), parents of those that dropped out had a lower education level and more often a non-traditional family structure. A larger bias was introduced by initially sampling in the municipality **Aalter**, a region with rather **high socioeconomic characteristics**. This has its implications on the validity of our results in obese and low socioeconomic populations. Low socio-demographic populations have been shown to have lower mental health (Reiss 2013), higher stress and cortisol levels (Evans and Kim 2013), unhealthier eating patterns (Patrick and Nicklas 2005) and a higher obesity prevalence (Shrewsbury and Wardle 2008). Due to this higher stress load, caution should be taken when using our percentile values for cortisol and HRV in population samples of lower socio-economic status. Related to this, the relation stress-cortisol might be different in lower SES samples. Stronger associations between stress and body composition might probably be found (1) in high-overweight populations due to stronger associations with food addiction / emotional eating (Moens and Braet 2007, Meule 2012) and the higher amount of fat mass and related stigma; and (2) in high stress populations due to more stress triggering.

Nevertheless, the large-scale approach and the use of **objective, biological stress measures** initiated the opportunity for **methodological papers** on anthropometric measures, percentile values, determinants of HRV, patterns and compliance in salivary cortisol sampling and comparison between stress biomarkers. This is one of the first studies examining the stress-adiposity relation in children while also considering biological stress measures and **different lifestyle factors**.

The chief advantage of our study is its **longitudinal design**. This design permits studying the directionality of the stress-adiposity relation. After all, the stress-lifestyle (Waladkhani and Hellhammer 2008, Gregory and Sadeh 2012, Stavrakakis, de Jonge et al. 2012) and stress-adiposity (Foss and Dyrstad 2011) hypothesis could be bidirectional. Although these bidirectional analyses might give more insight in potential causal relations, experimental studies are necessary to prove them. Observational studies still have the disadvantage of possible unmeasured confounding that can result in spurious predictor-outcome relations (effect explained by another characteristic than the expected predictor through its association with both exposure and outcome). Additionally, even longer follow-up studies would be necessary to study the effects of stress on obesity development in a non-clinical population.

Due to this longitudinal design, **elaborated statistics** were used correcting for relevant confounders: longitudinal mixed model analyses, percentile values with the LMS software, multilevel analyses in HLM software, cross-lagged analyses in Mplus software and tests for mediation and moderation.

3.2. Stress questionnaires

To cover the **different aspects of stress** (see definition in introduction and methodology), negative events, emotions and problem behaviour were examined by questionnaires. It allowed to study differential effects on endocrinology, lifestyle and adiposity. Apart from negative aspects, also some positive aspects have been included such as happiness and prosocial behaviour. Furthermore, we need to consider that the used psychometric questionnaires might not describe the whole psychological experience and that over- or underestimation is possible due to difficulties in collecting psychometric data of children.

Although we tried to standardize the interviewing conditions in the absence of parents and schoolteachers, there remain inherent problems in assessing complex concepts of chronic

stress among **young children**. Validated emotion self-report questionnaires almost exclusively exist for older children (starting around 9), therefore we opted for a short questionnaire with terminology comprehensible in infancy that showed moderate correlation (r=0.48) with the validated PANAS-C questionnaire. Further criticism is that the CLES questionnaire did not check the subjective appraisal of the stressors. Following our definition of stress (see chapter 1.1.), events only induce stress when they are perceived as stressful and when they exceed the individual coping resources. Also, the overall stress appraisal phase ("are you stressed?") was not measured. Lack of this information could thwart the tested relations of questionnaire data with biomarkers, lifestyle and adiposity. Therefore, future research should use life event questionnaires that also assess the subjective appraisal of events (for a recent review of such child questionnaires see (Vanaelst, De Vriendt et al. 2012)).

In addition, the questionnaires covered a relative short **time period** (last year's events, six month's difficulties, recent emotions). Since the recall ability of children is rather short in time, recalling over much longer periods might lead to an underestimation. Moreover, interviewers used calendar events such as birthdays, summer holidays and Easter to limit recall bias and to increase the accuracy of reporting in time (Garrison, Schoenbach et al. 1987). In using the CLES questionnaire, the event score of the last six months was used when analysing cross-sectional relations to obtain a similar time frame as in the SDQ. In the longitudinal analyses, the event score of the last twelve months was used since there was also a one year lag between the successive measurement waves.

Apart from different concepts, also different reporters were used. This **multi-informant** design with reports of both children and parents avoids informant-based biases (van Dulmen and Egeland 2011). Indeed, we found that children reported more emotional problems than their parents, especially in girls and older children. Interestingly, life events and emotional problems were significantly correlated only when using children's reported emotions (Michels, Vanaelst et al. 2012).

A related limitation is the **absence of parental mental health information**. After all, parental psychopathology might predict child psychopathology (Beardslee, Versage et al. 1998, Mendes, Loureiro et al. 2012), result in inaccurate reports of their child's problems (Ordway 2011) and might be associated with child adiposity (Ramasubramanian, Lane et al. 2013).

3.3. Stress biomarkers

Salivary cortisol was collected as marker of the HPA axis which has been hypothesised as a physiological link between stress and diet and adiposity (see section 4.2.1 and 4.2.2 of the introduction). Although cortisol sampling was an optional module within the ChiBS study, samples of 439 children were available (84%).

Salivary cortisol reflects the free plasma circulating cortisol. Apart from this active fraction in the blood, also active cortisol can be produced intracellular. The intracellular 11beta-hydroxysteroid dehydrogenase type 1 (11HSD1) converts inactive cortisone to active cortisol. Consequently, 11HSD1 can act as an amplifier of the glucocorticoid activity e.g. in the adipose tissue cells (Seckl and Walker 2001). Therefore, salivary cortisol might be an underestimation of the cortisol activity.

Participants were asked to collect saliva at **four time points over two days**, resulting in eight samples per person. By this repeated cortisol measure, several alternative parameters across the day could be considered such as the diurnal decline and the CAR. As was confirmed by the high cortisol intra-individual variability, more than two days of sampling could increase the reliability of our results, especially for the CAR (Hellhammer, Fries et al. 2007). Sampling for two days was chosen in this project since longer sampling could potentially lower protocol compliance and participation rate and raise laboratory costs.

Parents received a manual and checklist to ascertain **standardized procedures** of sampling (Adam and Kumari 2009). A quality control was executed by excluding morning samples collected more than 5 minutes different from the requested time point and evening samples not collected between 7 and 9 PM (271 out of 3290 samples). Furthermore, samples of corticosteroid-users were also excluded (5 children). A drawback was that only a subjective measure of **time compliance** was obtained (i.e. parental report) although devices for objective measurement of compliance are on the market. We stressed the importance of timing and the exclusion of self-reported non-compliers can already improve the accuracy (DeSantis, Adam et al. 2010). Nevertheless, it is probable that non-compliant people are the most likely to report their timing incorrectly, which could lead to missing a part of the morning increase and as such result in the observed low CAR (Kudielka, Broderick et al. 2003). Also, no information was available on sleep related confounders such as transient awakening, spontaneous versus forced awakening and the amount of light in the sleeping room (Scheer and Buijs 1999, Raikkonen, Matthews et al. 2010).

Salivary cortisol was **only collected at baseline** because of high costs, high burden for the participants and logistics. As a result, no bidirectional relations could be examined.

HRV was collected as a quantitative marker of the autonomic nervous system (1996). Standardized short-term HRV measurements with an extra manual **quality control** and model selection were executed in a large healthy child population of 460 subjects. These measures were performed in **supine position**. Since supine position could diminish sympathetic activity, future research should also test basal HRV measures in sitting position as marker of chronic stress (Vybiral, Bryg et al. 1989). Although visual respiratory observation was done during the registration, **breathing rate** was not measured with an extra channel. Breathing rate was shown to be negatively related with LF and HF power, but had no influence on time-domain parameters (Brown, Beightol et al. 1993, Penttila, Helminen et al. 2001). Nonlinearities are more likely to happen with a low breathing rate (e.g. at 10/minute breathing rate), a long RRI and in the presence of important respiratory sinus arrhythmia (Porta, Baselli et al. 2000). Nevertheless, the influence of nonlinearities will be minimal in our population as children have a general high breathing rate (18-21/minute) and short RR-interval; and respiratory sinus arrhythmia induced by the Valsalva manoeuvre was more likely abolished.

In the last years, **technology advanced rapidly** and innovative devices have been introduced that can measure several parameters simultaneously and that are also much smaller to increase the wearing comfort. For instance, the Actiheart (CamNtech Ltd U.K.) device combines an ambulatory HRV measure with physical activity measures. Other devices from the same manufacturer such as the Actiwave also record sleep stages.

Although HRV reflects the autonomic activity, it mainly comprises the parasympathetic activity. Other parameters for this stress axis have been recently established such as the salivary **alpha-amylase** concentration that is reflecting the sympathetic activity (Granger, Kivlighan et al. 2007). Collecting both HRV and alpha-amylase would result in more detailed information.

Both salivary cortisol and HRV were measured at a basal, unstimulated level. Perhaps chronic stress does not always lead to chronic hyper/hypocortisolism patterns, but rather influences stress reactivity to future stressors (i.e. the intensity of physiological response in HRV and stress levels). Therefore, future studies should also consider HRV and cortisol **stress**

reactivity in the stress-lifestyle and stress-adiposity relation. Some results in literature already show stress-induced sedentary and activity behaviour differences depending on HRV reactivity (Balantekin and Roemmich 2012) or stress-induced snacking differences depending on cortisol reactivity (Epel, Lapidus et al. 2001, Newman, O'Connor et al. 2007). High cortisol reactivity was also indicated as a mediator between girls' depression and overweight (Dockray, Susman et al. 2009). Although physiological hyperreactivity is sometimes hypothesized, also a blunted stress reactivity could be plausible (Phillips 2011). Consequently, more research in this area is needed.

3.4. Lifestyle

A **multiplicity** of lifestyle factors was examined: diet, eating behaviour, physical activity, sedentary behaviour and sleep duration. Since parental report was used for most lifestyle factors, a reporting bias may be introduced: parents may have the tendency to give the morally right answer or may inaccurately estimate their child's lifestyle behaviour.

Diet was of main interest in this study, because of its important role in the development of adiposity. First, the general food pattern was examined using a FFQ. Although we had an elaborated measurement of dietary pattern focussing on several aspects (sweet foods, fatty foods, snacks, fruit & vegetables), **no quantitative information** on total dietary or nutrient intake nor portion sizes was collected via the FFQ. Nevertheless, using a FFQ has the advantage of showing the habitual diet as dietary recalls can be biased by exceptional days.

Since the children were too young to report dietary intake themselves, our instrument still has the restriction that only parental report is available. After all, parents do not always have the overall picture of their children's food intake. Although they generally can find out what is on the menu of the child's school (on the school's website), they can only guess whether their child has eaten all ingredients and whether an unhealthy snack/dessert was chosen. This could result in under- or over-reporting (Byers, Treiber et al. 1993, Roumelioti and Leotsinidis 2009). Since the responder burden was already high, no information was collected on **parental control** over diet or parental dietary habits. Yet, children's diet is still highly under parental control. Although stress has been shown to influence children's diet in laboratory studies where there is no influence of parents (Roemmich, Wright et al. 2002, Roemmich, Lambiase et al. 2011), it remains important to examine or account for the parental influence in real-life studies. Emotional eating of the parent and diet-related parenting styles have been

shown to increase the child's emotional eating (Kroller, Jahnke et al. 2013). Even more important, parental stress was able to increase the children's fast food intake and decrease their vegetable consumption (Renzaho, Kumanyika et al. 2011, Parks, Kumanyika et al. 2012).

Secondly, eating behaviour was examined using the **DEBQ**. An advantage of this questionnaire is the child self-report. The DEBQ is not only focussing on the factual act of eating in the absence of hunger, but especially on **intentions to eat** in those situations. As non-overweight children might more closely control their desire (Moens and Braet 2007), this could explain the absence of a relationship between emotional eating behaviour and the actual diet in our low-overweight group.

Apart from questionnaires on physical activity and screen time, activity was also measured objectively with **accelerometers** worn on the hip, both in 2010 and 2012. This allowed to register sedentary and activity behaviour as well as the intensity of physical activity. After all, reported screen time is only a fraction (although an important one) of children's sedentary behaviour and questionnaire data do not capture all ways of being physically active. The accelerometer was also used around the wrist at night to record **sleep quality**: arm movements for a period of time are then scored as awake. As such, the sleep efficiency could be calculated. Although the accelerometer is a valid objective measure of sleep quality with low cost and invasiveness (Sadeh 2011), polysomnography remains the golden standard. Besides, sleep quality was only measured at baseline due to logistic restraints. Between the start of the ChiBS study and now, more advanced devices for sleep quality measurements at home have become widely available e.g. the Actiwave® (CamNtech Ltd U.K).

3.5. Adiposity

One of the major strengths of this project is the large-scale use of **ADP technology** for precise fat% determination. As one of the best performing two-compartment methods, ADP is considered as a more feasible method for large scale surveys in comparison with the four-compartment model (Fields and Goran 2000). A disadvantage of ADP remains the lack of information on fat distribution. Therefore, also routine anthropometric measurements such as the waist circumference were conducted by three trained researchers to minimize inter- and intra-observer variability. Since only a 7-8% overweight prevalence existed in our population, adiposity was used as a continuous variable not as a categorical variable.

The **multifactorial aetiology** of obesity hampers studying the stress effects on adiposity on population level. After all, stress is only one of the many factors that can stimulate fat deposition. Although we considered age, sex, lifestyle and socio-economic status, the ecological model of obesity predictors also stresses the importance of ecological factors (family, community and society) and genetics. More than 100 candidate genes have been found amongst others those influencing appetite hormones like leptin. Also genetic differences in cortisol levels (Van Hulle, Shirtcliff et al. 2012), the cardiovascular stress response (Wu, Snieder et al. 2010) and the epidemiology of mental health (Danese 2008) have been demonstrated.

4. Future research

A summary of some important ideas for future research in the area of the stress-adiposity relation in children is visualized in Figure 37. Our results stressed the importance of **longitudinal** studies since some relations may perhaps be bidirectional. These **large-scale** longitudinal studies ideally are **interdisciplinary** to cover both the biological and psychological aspects. They should also use **multiple methods** to examine all the relevant determinants and outcomes e.g. objective measurements of stress and lifestyle are necessary next to the self-reports. Moreover, several aspects of stress need to be considered. Nevertheless, also **experimental studies** are warranted to further prove the suggested causalities. Besides, several other **methodological** hints for future research were already mentioned in our methodological considerations: measuring perceived stress, stress reactivity, alpha-amylase measurements, objective cortisol sampling compliance, info on parental mental health and lifestyle control, sleep quality measurements, advanced technology to combine different measures (e.g. HRV, activity and sleep), quantifying the food intake and the determination of fat distribution.

Most imperative, our findings should be **verified in a more representative population sample** including obese and low socio-economic participants. Apart from recruiting in regions with general low socio-economic characteristics, efforts can be done in recruitment by translating letters, working with interpreters, using personal contact (face-to-face or combining different approaches), offering financial and medical benefits, incorporating flexibility in time and location, offering free transportation and increasing their awareness of the study's impact (Ejiogu, Norbeck et al. 2011). A recent study concluded that minority parents were as likely to consent their child's participation when they were asked by their own

physician (Reiss 2013). This indicates the importance of collaboration with health professionals and community members to enhance trust. Since 10% of the Flemish children lives in a household with an income under the national poverty risk threshold (Studiedienst Vlaamse regering 2013), questions on financial related stress should be included in the questionnaires.

In the next paragraphs we explore new hypotheses that could be considered in future research. (1) The predictor stress can be considered in a life perspective: **perinatal stress** could already lay an important foundation for future health. (2) The outcome of stress could even be looked at broader by considering the whole **metabolic syndrome**. (3) Finally, some underlying aspects need further attention such as **appetite hormones** and **reward sensitivity**. Also other mechanisms that have been linked with both stress and adiposity may be further explored such as microbiota composition and activity (Rhee, Pothoulakis et al. 2009, Tagliabue and Elli 2013). Overall, the main challenge is to identify biological and behavioural correlates of adversities and of the person's resilience or vulnerability to this health deteriorating allostatic load.

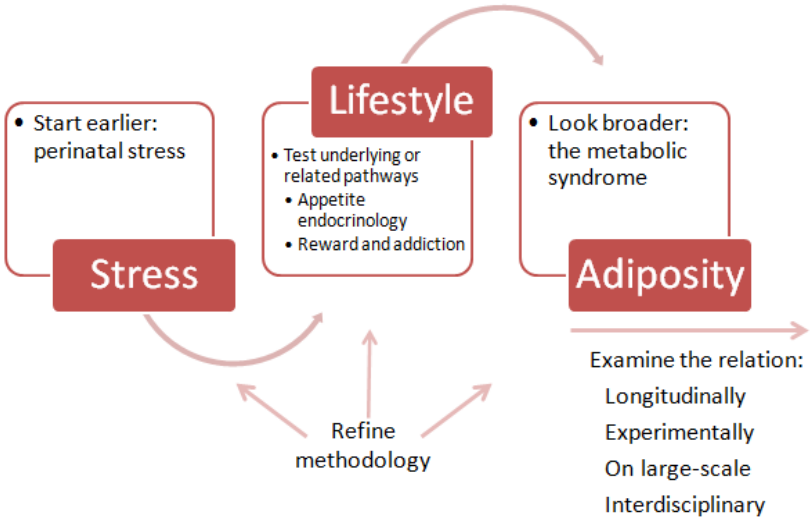


Figure 37: Aspects for future research in the stress-adiposity relation in children

4.1. Perinatal stress

Experiences of stress already start in the perinatal life. Examples of such experiences are maternal stress, nutrient restriction, growth restriction and intra-uterin toxin/pharmaceutical exposure, but also include parental neglection and infections postnately. Maternal stress can increase cortisol levels in the mother and subsequently enhance fetal cortisol exposure. This impacts cortisol stress reactivity by effects on the HPA and limbic development and function

(e.g. receptor levels that finally determine the negative feedback) (Matthews 2002) but it also increases ANS reactivity (Kajantie and Raikkonen 2010) leading to overall **excessive stress responsitivity**. Moreover, prenatal stress affects hormonal regulation of **hunger and satiety** (leptin, ghrelin, insulin) and **adipogenesis** (partially due to epigenetic changes) (Entringer, Buss et al. 2010). In conclusion, perinatal stress may instigate increased susceptibility to stress-induced adiposity. Consequently, longitudinal **birth cohorts** starting at pregancy or even before conception are warranted. They can utilize a life-course perspective on stress-adiposity by examining the role of prenatal stress and its effects on later life stress reactions and adiposity.

4.2. Metabolic syndrome

Continuous high cortisol does not only stimulate fat deposition. It could also account for **inflammation**, high **blood pressure**, **insulin resistance**, accelerated **blood coagulation** and disturbed **blood lipid** levels (e.g. cholesterol). All these complications fit together in the metabolic syndrome that finally can lead to cardiovascular diseases and diabetes (Bjorntorp 2001). Stress-induced metabolic changes could be one of the mechanisms that increase metabolic syndrome both (1) directly due to its interaction with the ANS, growth, appetite, metabolism and immune axes and (2) indirectly through stress-induced adiposity (Charmandari, Tsigos et al. 2005, Pervanidou and Chrousos 2011). Importantly, the cortisol activating enzyme 11β-hydroxysteroid dehydrogenase-1 is present at several tissue levels that are associated with the metabolic syndrome: the liver, pancreas, muscles, vasculature and adipose tissue (Anagnostis, Athyros et al. 2009, Cooper and Stewart 2009). In summary, it would be of interest that studies on the effects of stress should consider the broad outcome of metabolic syndrome, after correction for other metabolic predictors.

4.3. Hormonal pathways in appetite

More research is needed on the detailed hormonal pathways in the stress-adiposity relation. This may provide potential targets for treatment of obesity in people with an overactive HPA axis.

It is hypothesised that cortisol influences reward and appetite by upregulating **NPY** (increased appetite and reward) and dysregulating **insulin** and **leptin** levels (the body becoming resistant to their appetite and reward reduction) (Adam and Epel 2007). Recent literature also suggest a

stress induced change of other hormones like **ghreline** (orexigen and mood-regulating) (Chuang and Zigman 2010, Perello and Zigman 2012, Schellekens, Finger et al. 2012) and **adiponectin** (anti-inflammatory, insulin-sensitizing, energy-regulating and possibly anti-depressant) (Dridi and Taouis 2009, Liu, Guo et al. 2012). Based on these suggested mechanisms, we aim to have information on adiponectin, ghrelin and leptin levels in the ChiBS participants in order to examine their association with cortisol as well as their role in the stress-diet and the stress-adiposity relation in the future.

4.4. Brain reward and addiction pathways

Psychological behavioural characteristics have been linked with the development, maintenance and treatment resistance of obesity, in particular related to the high food intake. First of all, obese children are **more cue-responsive**: they are more vulnerable to food cues and consequently will react to them by eating the food (Jansen, Theunissen et al. 2003). Obese children have also been shown to be **more impulsive** than the lean children: they find it more difficult to resist food intake (Nederkoorn, Braet et al. 2006). This is reflected in **enhanced disinhibition** (the lack to control inhibition) and **enhanced reward sensitivity** (a high motivation to strive for immediate reward despite punishment rather than waiting for a delayed but larger reward).

These findings encourage the study of these disinhibition and reward concepts in the stress-diet relation. Is there a co-existence with stress? Might they act as moderators in the stress-diet relation? Can they explain personal differences in stress-induced eating (more versus less intake)? Very recently, some preliminary evidence has been published that stress-reactive individuals show diminished sensitivity to reward, but not punishment, under acute stress (Berghorst, Bogdan et al. 2013). The question remains what the effects of chronic stress are on reward. A possible biological mechanism linking these concepts with stress is the physiological interaction of stress with the mesocorticolimbic dopaminergic reward system (Charmandari, Tsigos et al. 2005, Trainor 2011). These interactions with the reward/motivation pathways and dopaminergic signalling squeak parallelisms with **addiction** pathways (Brownell and Gold 2012, Sinha and Jastreboff 2013). After all, the increased salience of comfort food induces a conditioning with greater wanting and seeking of these foods. In animal studies, the same brain regions were involved in food cravings as in drug cravings and food restriction induced cortisol increments that lead to this addictive

conditioning of greater food seeking (Guarnieri, Brayton et al. 2012). These findings initiated the terminology 'food addiction' (Pelchat 2009, Brownell and Gold 2012). In an obese sample of children and adolescents, 29% reported themselves as addicted to food (Pretlow 2011). Interestingly, cross-sensitization of food and addictive substances (drugs, alcohol, nicotine) might happen, resulting in a co-occurrence of addiction and stress/psychiatry (Avena, Rada et al. 2008, Brownell and Gold 2012).

In summary, the links with brain reward and addiction pathways advocates the integration of **neuroimaging** (e.g. activity in the dopaminergic centra) **and behavioural research** (e.g. behavioural tasks) in stress-adiposity research. Finally, this could shed light on new scientific perspectives and as such lead to new treatment alternatives.

5. Public health perspectives

Overall, the results revealed childhood stress as an important determinant for hormonal changes and longitudinal lifestyle changes that further might stimulate adiposity. Since both stress and obesity account for a significant proportion of the global burden of disease, prevention strategies should be established.

5.1. Strategies to tackle the stress-adiposity relation

Strategies to tackle the stress-adiposity relation can be generated on different levels and different aspects as shown in Figure 38.

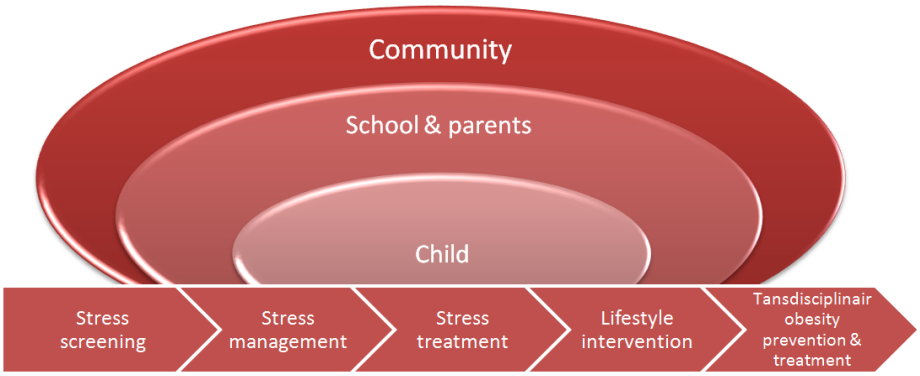


Figure 38: Aspects and levels in tackling the stress-adiposity relation in children

A first aspect is to **screen** for the presence of psychosocial stressors (e.g. traumatic events; for a practical review see (Strand, Pasquale et al. 2010)) and symptoms. As such, high-risk children can be identified and monitored. A second step is training the **management of stress** to make children capable to cope with stress. A logical third step when stress levels are increased implies the **treatment of stress** (Everly and Lating 2013). Apart from pharmacological treatment, also cognitive behavioural therapy, relaxation techniques (mediation, respiratory control and neuromuscular relaxation), hypnosis, biofeedback and even physical activity have been applied. A fourth step is to intervene in stress-associated **lifestyle** factors such as emotional eating and decreased physical activity. When obesity has been developed yet, a final step is the **transdisciplinairy obesity treatment** with a special focus on psychological support.

All these aspects can be tackled at several levels. Some aspects of intervention preferentially take place at the **individual** level e.g. personalized psychotherapy. Nevertheless, most interventions are school interventions since the school is an easy way to reach all children at once with adult role models and the social network of peers. The **school** based intervention can be comprehensive (adapting the school environment and involving the parents and community) or curriculum-based. Apart from school interventions, the **parents or family** remain important actors in the child's environment to target both stress and lifestyle. Also more universal **community** intervention programs (outside school or family) belong to the possibilities. The internet-based interventions constitute an important promising subgroup (Rose, Buckey et al. 2013).

Since stress is not inevitable, focus should be laid on **stress management** skills. Prevention in children ought to create a firm basis and transferable skills for further life. As stated above, parents have a special role to play in the education of their kids by being a role-model and by their parenting style. Therefore, parenting support training in group can focus on interpersonal warmth, family activities, responsiveness and assertive parenting skills with strict rules that are age-appropriate (Desai 2010). Effective childhood stress interventions are tailored (to culture, age and sex), evidence-based, long term, have a holistic approach, involve the children themselves and use competent actors (Adi, Killoran et al. 2007, VIGeZ 2011). Tailoring to kids can be achieved by using face pictures for stress expression and cartoons or

power hero cards for skills training (Macklem 2008). Some potential stress management methods compromise (Desai 2010):

- Self-awareness and self-esteem
- Pro-active thinking skills (in contrast to rigid thinking of 'must' and 'should')
- Emotional regulation
- Social development of relationships (respect, positive feelings, conflict management)
- Problem solving skills or coping techniques

Resilience is a related terminology that may be thought of as the characteristic or ability to positively adapt to and/or rebound from significant adversity and distress. Everly summed up seven important lessons that children should learn to attain high resilience: social support, courage, responsibility, caring for oneself, optimism, faith and integrity (Everly 2009).

Lifestyle was a moderator in the stress-adiposity relation. Consequently, stress-induced lifestyle changes have to be targeted. After all, lifestyle behaviour can track from childhood into adulthood (Thorleifsdottir, Bjornsson et al. 2002, Mikkila, Rasanen et al. 2005, Ashcroft, Semmler et al. 2008, Telama 2009). The transition period of childhood is therefore a critical phase for these interventions. To prevent overweight, the environment (e.g. at home, at school etc.) needs to be an 'activity encouraging, healthy food zone' that minimizes opportunities for stress-induced eating but maximizes opportunities for physical activity. **Emotional eating** is an easy choice in our food-abundant environment. This form of hedonic eating (eating for pleasure rather than for energy needs) is further stimulated by changes in the social norms on food since there is substantial encouragement to eat in all circumstances and few prohibitions against doing so (Lowe and Butryn 2007). Therefore, parents and children should be made aware of this stress-induced eating and problem-solving coping skills should be highlighted as an alternative for coping stress by food consumption (Birch and Davison 2001, Kroller, Jahnke et al. 2013). Emphasis should be put on the contextual and especially familial environmental factors of children's dietary behaviour. Parents should make an example by their own eating style and by lowering the availability of unhealthy food. Also, parental stress should be treated as a risk factor since it can directly and indirectly enhance children's fast food consumption and lower their vegetable consumption (Renzaho, Kumanyika et al. 2011, Parks, Kumanyika et al. 2012).

Concerning **physical activity**, a review reported that young people have a consistent desire to be active but that they are often constrained by external factors such as school policy or curricula, parental rules in relation to safety and convenience, and physical environmental factors (Dollman, Norton et al. 2005). Since physical activity is a perfect reaction to stress (Tsatsoulis and Fountoulakis 2006), physical activity must be encouraged and long screen time must be discouraged.

Relaxation and mindfulness **interventions** to reduce emotional eating have revealed mixed results in adults (Manzoni, Pagnini et al. 2009, Daubenmier, Kristeller et al. 2011, Kearney, Milton et al. 2012). A recent meta-analysis indicated that intervening in lifestyle such as diet and physical activity is less efficacious than intervening in stress management on itself and the efficacy of interventions overall increased from childhood and adolescence through late middle age (Johnson, Scott-Sheldon et al. 2010). Consequently, intervening in inter alia emotional eating in children will be a though problem to deal with in the future.

Since these stress-adiposity relations might be **bidirectional**, the weight of stressed people should be monitored, while mood should be monitored in overweight patients. This is in agreement with a broader initiative focusing on the challenges and potentials in **transdisciplinary obesity research** (Holm, Børker Nielsen et al. 2013).

5.2. Initiatives to manage and prevent stress

Several initiatives to manage and prevent stress exist. Some of them are shortly introduced here.

On global level, the **WHO** released their mental health action plan for 2013-2020. In their plan, they also focus on children's mental health with action recommendations on emotion management, social relationship building and positive sense of identity. Apart from promotion/prevention programmes, they also emphasize to scale-up **mental health services** (WHO 2005, Chisholm, Flisher et al. 2007).

In Belgium, psychosocial aspects are increasingly assimilated in the **educational curriculum** of kindergarten and primary school children (AKOV 2010). Concepts such as respect, conflict handling, emotional expression, behaviour, self-confidence, asking for help and time planning are already integrated in the study domains language, physical education and world

orientation. In primary school, there also exists a domain exceeding theme on social skills that focuses on inter-personal relations.

Different **information channels and initiatives** are also available in our country. Most importantly, there is a Flemish society for mental health "Vlaamse Vereniging voor Geestelijke Gezondheid". Their website "www.geestelijkgezondvlaanderen.be" is an informational platform that informs people on the topic and guides them through the health care services and other initiatives. A second player are the health insurances that provide additional reimbursement for psychotherapy in children and adolescents (Steunpunt Jeugdhulp 2011) and that also start information campaigns and websites (often in collaboration with the governmental organisations). One example is "www.joetz.be/stresskip", a website for children on stress including some tests and tips. Another example of such a website was initiated by the Flemish institute for health promotion (VIGeZ): "www.noknok.be" is a website on mental well-being for adolescents with tips, exercises but also polls, testimonies and videos. A different approach are frisky theatre performances for youth on depression (Te Gek Intiem) or stress and fear of failure (Ikandani). Nevertheless, these performances often aim at secondary school children. Finally, there are telephone lines for help such as 'Awel' the line for children and youth with problems and the 'opvoedingslijn' a telephone line for parents with educational questions.

In summary, there are already some promising initiatives. The challenge will be to **maintain the interest**. The existing initiatives should be kept running, additional large-scale and scientific-based initiatives should be started and the integration into daily life and schools should be encouraged. Importantly, children should be guided to these initiatives and should have the courage to search or ask for help. Most initiatives focus on adolescents, thus **more attention is needed for the younger age group of primary school children**. Related to this, detailed national representative numbers on children's stress level are lacking since the national health survey only included participants older than 14y old up to now. While the existing initiatives mainly focus on the management and prevention of stress, an additional focal point should be the **stress-induced lifestyle** changes such as emotional eating and sedentarism. After all, the link between stress and physiological health is still underestimated.

VII.

SUMMARY

Background

The last decades have been characterized by a global growing **overweight epidemic**. Most alarming is the increase in childhood overweight with prevalence ranges between 10 and 40% in European countries. Excessive caloric intake, insufficient physical activity and sleep deprivation are major lifestyle factors involved in the development of childhood overweight. Another recently identified potential predictor of overweight is **chronic stress**.

Some evidence on the stress-lifestyle-adiposity relation exists but the directionality is still unclear. Therefore, **longitudinal studies** are warranted. Recent reviews also highlighted the lack of evidence in the young age groups such as **primary school children**. Although children are not always recognized as being susceptible to stress, chronic exposure to stressful situations is not uncommon and may adversely affect their physiological and psychological health. After all, childhood is a period of incessant physical and brain development creating the foundation of adult psychopathology, lifestyle and adiposity.

Therefore, we started a longitudinal study in a population sample of (non-clinical) children on the stress-adiposity relation. The **hypothesis** was that chronic stress may promote overweight directly through hormonal increase of fat deposition and/or indirectly through changes in lifestyle factors. Expected lifestyle changes were an increased consumption of energy dense highly palatable (sugar and fat rich) foods and a deviant eating behaviour, decreased quantity and quality of sleep and a decreased amount of physical activity with an increase in sedentary screen time.

Methods

The **ChiBS study** (Children's Body composition and Stress) is an observational cohort study that was designed to investigate the relationship between chronic psychosocial stress in young children (5-12 years old) and changes in body fat over a two-year follow-up period (2010-2012). Enrolment of participants for the baseline ChiBS survey was integrated in the ongoing European sixth framework programme IDEFICS by recruiting children from the Belgian IDEFICS control region i.e. the municipality Aalter. At baseline, 523 children participated in the study of which 453 participated again in 2011 and 330 in 2012.

Major **study parameters** were stress, lifestyle and adiposity. Stress was measured with questionnaires on children's negative events, emotions and behavioural problems. Moreover, two biological stress markers were collected to represent the major physiological stress

systems: salivary cortisol representing the hypothalamic-pituitary-adrenal system and heart rate variability (HRV) representing the autonomic nervous system. Body composition was measured with routine anthropometry (i.a. weight and height and waist circumference) and a fat percentage determination with the BOD POD device based on air-displacement. Dietary pattern, psychological eating behaviour, physical activity (report and objective measure), sedentary behaviour (report and objective measure) and sleep (duration and quality) were considered as important lifestyle parameters.

The first **goal** was to test the feasibility and interrelationships of the different stress measurements (reports and physiology) in children. The second and main goal was to examine the influence of chronic stress on the evolution of adiposity parameters longitudinally, taking into account lifestyle factors and endocrinology as intermediary factors in this relationship. Understanding the mechanisms by which stress may predispose to overweight, will elucidate preventive strategies.

Results

The first **methodological papers** on stress revealed that (1) HRV measurements should be corrected for age, sex and physical activity/fitness when being used as stress indicator and that (2) salivary cortisol measurements should be corrected for age and awakening time after excluding time noncompliers. Moreover, percentile values for HRV and salivary cortisol were presented. The methodological analysis on body composition determination suggested the use of triceps and subscapular skinfold sum as best alternative in the absence of more sophisticated technologies like air-displacement.

A first goal was to test the **interrelationships of different stress measures**. The relation between psychometric data and cortisol levels predominantly supported a cortisol stimulating effect of stressors in children. The relation between psychometric data and HRV levels predominantly supported a decrease in parasympathetic activity. Interestingly, differential associations were seen: HRV was mainly associated with negative emotions, while cortisol was mainly associated with negative events and lower happiness. This differential association may indicate activation differences in the systems underlying both biomarkers. Nevertheless, HRV and cortisol showed mutual moderate associations. Consequently, both HRV and cortisol can be used as valid stress marker but measuring both pathways stays recommended as they might be stimulated differently depending on the stressor or stress outcome.

A second goal was to examine the **influence of stress on lifestyle and adiposity**.

Diet was the most intensively examined lifestyle parameter. Salivary cortisol patterns that might reveal higher stress levels were associated with an unhealthier dietary pattern: higher fatty food and snack consumption frequency, but especially higher sweet food consumption. These results suggest the cortisol-induced comfort food preference that might explain the relation between stress and an unhealthy diet. Indeed, stress was related with longitudinal maladaptive eating behaviour (higher emotional, external and restrained eating) and dietary intake (sweet food consumption frequency). The relations were depending on the used stress construct and children's age and sex. Sweet food consumption was only increased in older children, emotional eating only in girls, external eating mainly in girls and restrained eating only in boys and older children. Remarkably, maladaptive eating behaviours could also increase anxiety feelings.

A second examined lifestyle parameter was activity. Both objective and reported physical activity were longitudinally augmented by high stress. Nevertheless, this relation was different depending on the age of the children: negative emotions increased activity in the youngest group, but decreased activity in the oldest group. No longitudinal relations were found for measured sedentary behaviour and reported screen time.

A last examined lifestyle parameter was sleep. Short sleep duration but not low sleep quality was cross-sectionally associated with high stress (mainly negative emotions). Nonetheless, no longitudinal relations were detected for sleep.

Although no direct longitudinal effect of stress on adiposity was detected, lifestyle and cortisol moderated this relation leading to positive stress-adiposity associations in those with high cortisol patterns and an unhealthy lifestyle (frequent sweet food consumption). Also a relation in the opposite cause-effect direction existed: body composition influenced stress levels since children with a higher body mass index or fat% reported stronger negative emotions one year later.

Discussion

Overall, the results revealed **childhood stress** as an important determinant for hormonal changes and longitudinal lifestyle changes (mainly an unhealthy diet and eating behaviour) that further might stimulate overweight development. Nevertheless, individual differences (e.g. by age and sex) have been found in these hormonal and lifestyle reactions to stress. These differences will partly determine the child's adiposity risk since cortisol and lifestyle moderated the stress-adiposity relation. Consequently, both the endocrinological pathway and

the lifestyle behavioural pathway in stress-adiposity may be important. Longer follow-up studies will be necessary. As children grow older, the lifestyle pathways will probably become even more important because adolescents will have more independence over food choices and more time constraints for performing physical activity. Importantly, the stress-adiposity relations appeared to be bidirectional since adiposity and eating behaviour also influenced some stress aspects on the longitudinal perspective.

Since both stress and overweight account for a significant proportion of the global burden of disease, **prevention strategies** should be established.

Strategies to tackle the stress-adiposity relation can be generated on different levels and different aspects. After all, stress screening, stress management, stress treatment, lifestyle intervention and obesity treatment are essential aspects. Stress management can focus on skills such as problem-coping skills and emotional regulation while also positive aspects such as social support and self-esteem can be targeted. Concerning lifestyle, the environment need to be an 'activity encouraging, healthy food zone' that minimizes opportunities for stress-induced eating but maximizes opportunities for physical activity. Since these stress-adiposity relations might be bidirectional, the weight of stressed people should be monitored but also mood should be monitored in overweight patients covering the idea of transdisciplinary overweight treatment. All these initiatives can be operationalized at the child level, family level, school level and community level. Since most existing initiatives focus on adolescents, the attention should be redirected to the younger age group of primary school children.

Finally, suggestions were formulated for **future research** in high risk populations (high obese or low socio-economic populations), methodological improvements, the role of contextual influences (e.g. parents), the use of a life perspective by considering perinatal stress, broadening the outcome to the whole metabolic syndrome and further elaborating underlying aspects such as appetite hormones and reward sensitivity.

VIII.
SAMENVATTING

Achtergrond

Gedurende de laatste decennia kent de **overgewichtepidemie** een globale opmars. Vooral de toenemende prevalentie van overgewicht bij kinderen (tussen de 10 en 40% voor Europese landen) is alarmerend. Overmatige energie-inname, onvoldoende lichaamsbeweging en slaaptekort zijn de belangrijkste leefstijlfactoren die zijn betrokken bij de ontwikkeling van overgewicht. Een andere recent geïdentificeerde determinant van overgewicht is **chronische stress**.

Er is reeds enige evidentie voor de relatie tussen stress, leefstijl en overgewicht maar de oorzaak-gevolg richting is nog steeds onduidelijk. Bijgevolg zijn **longitudinale studies** vereist. Recente artikels wijzen vooral op het gebrek aan bewijs bij jonge leeftijdsgroepen zoals **kinderen uit de lagere school**. Alhoewel kinderen niet altijd aanzien worden als stressgevoelig, is chronische blootstelling aan stressvolle situaties niet zeldzaam en kan dit hun fysiologische en psychologische gezondheid schaden op korte en/of lange termijn. De kindertijd is immers een periode van continue hersenontwikkeling en fysieke ontwikkeling. Deze ontwikkelingen hebben een invloed op de psychopathologie, leefstijl en lichaamssamenstelling in het latere, volwassen leven.

Om deze redenen werd een longitudinale studie opgestart rond de relatie tussen stress en overgewicht in een niet-klinische populatiesteekproef van kinderen. De **hypothese** was dat chronische stress op een directe wijze overgewicht kan bevorderen via hormonale stimulatie tot vetopstapeling en/of op een indirecte wijze door veranderingen in leefstijl. De veronderstelde leefstijl veranderingen waren een verhoogde inname van voedingsmiddelen met een hoge energiedichtheid (rijk aan vet en suiker), een ongezond eetgedrag, verminderde slaapduur en slaapkwaliteit en verminderde lichaamsbeweging met een verhoging in sedentair gedrag bijvoorbeeld door schermblootstelling (televisie en computer).

Methoden

De **ChiBS studie** (Children's Body composition and Stress) is een observationele cohortstudie die de relatie beoogt te onderzoeken tussen chronische, psychosociale stress bij jonge kinderen (5 tot 12 jaar) en veranderingen in lichaamsvet over een twee jaar durende opvolgperiode (2010-2012). Rekrutering van deelnemers was geïntegreerd in het lopende, Europese 6de kaderprogramma IDEFICS door kinderen aan te schrijven uit de Belgische

IDEFICS controleregio (de gemeente Aalter). Bij de start namen 523 kinderen deel waarvan 453 opnieuw in 2011 en 330 in 2012.

De belangrijkste **studieparameters** waren stress, leefstijl en overgewicht. Stress werd gemeten met vragenlijsten over de negatieve gebeurtenissen, emoties en gedragsproblemen van de kinderen. Verder werden ook twee biologische stressmerkers gemeten om de voornaamste fysiologische stresssystemen te bestuderen: speekselcortisol vertegenwoordigt de as hypothalamus-hypofyse-bijnier en hartslagvariabiliteit (HRV) vertegenwoordigt het autonome zenuwstelsel. Lichaamssamenstelling werd gemeten via routineantropometrie (gewicht, lengte en buikomtrek) en vetpercentage werd gemeten met een BOD POD-toestel dat werkt volgens het principe van luchtverplaatsing. Het voedingspatroon, psychologisch eetgedrag, lichaamsbeweging (gerapporteerd en objectief gemeten), sedentair gedrag (gerapporteerd en objectief gemeten) en slaap (kwaliteit en kwantiteit) werden beschouwd als leefstijlfactoren.

Het eerste **doel** was om de haalbaarheid en de samenhang te testen van verschillende stressmetingen (via rapportage en fysiologisch) bij kinderen. Het tweede doel en tevens het hoofddoel bestond erin de invloed van chronische stress te meten op de longitudinale evolutie van lichaamssamenstelling, waarbij rekening gehouden werd met leefstijl en hormonale veranderingen als intermediaire factoren. Een beter begrip van mechanismen in door stress geïnduceerde vetopstapeling zal immers bijdragen tot het ontwikkelen van preventieve strategieën.

Resultaten

De eerste **methodologische artikels** rond stress toonden aan dat (1) HRV metingen als stressindicator gecorrigeerd dienen te worden in functie van leeftijd, geslacht en fysieke activiteit/fitheid en dat (2) speekselcortisolmetingen gecorrigeerd dienen te worden voor leeftijd en ontwaaktijd na exclusie van stalen die niet afgenomen werden binnen de vooropgestelde tijd. Verder werden percentielwaarden voor HRV en speekselcortisol bepaald. De methodologische analyse van lichaamssamenstelling suggereerde het gebruik van de triceps en subscapulaire huidplooimetingen als het beste alternatief in de afwezigheid van meer gesofisticeerde technologieën zoals de BOD POD.

Een eerste doel was om de **samenhang tussen verschillende stressmetingen** te verifiëren. De relatie tussen psychometrische data en cortisolniveaus wees voornamelijk op een cortisol stimulerend effect van stressoren bij kinderen. De relatie tussen psychometrische data en

HRV-waarden wees voornamelijk op een daling in parasympathische activiteit als gevolg van stress. Beide merkers toonden echter een relatie met andere psychometrische data: HRV was vooral geassocieerd met negatieve emoties terwijl cortisol vooral geassocieerd was met negatieve gebeurtenissen en verminderde blijdschap. Deze verschillen duidden mogelijk op een activatieverschil in de beide fysiologische systemen. Er werd wel een matige associatie gevonden tussen HRV en cortisol onderling. Bijgevolg kunnen cortisol en HRV als valide stressmerkers gebruikt worden, maar in het ideale geval zouden beide merkers gemeten worden daar ze op een andere wijze kunnen gestimuleerd worden afhankelijk van de stressor of de stressuitkomst.

Het tweede doel bestond erin de **invloed van stress op leefstijl en overgewicht** na te gaan. Voeding was de meest intensief onderzochte leefstijlparameter. Speekselcortisolpatronen die een hoger stressniveau weergeven waren geassocieerd met een ongezonder eetpatroon: hogere consumptiefrequentie van vetrijke voeding en snacks en vooral van zoete voedingsitems. Deze resultaten suggereren de cortisol geïnduceerde voorkeur voor ‘comfortvoedsel’, wat op zijn beurt de relatie tussen stress en een ongezond voedingspatroon verklaart. Stress was inderdaad longitudinaal geassocieerd met maladaptief eetgedrag (meer emotioneel, extern en lijngericht eten) en ongezonde voedingsinname (hogere inname van zoete voedingsmiddelen). De relatie was afhankelijk van het gebruikte stressconstruct, leeftijd en geslacht van de kinderen. De verhoogde consumptie van zoete voeding door stress werd alleen gezien bij de oudere kinderen, emotioneel eten alleen bij meisjes, extern eten vooral bij meisjes en lijngericht eten alleen bij jongens en oudere kinderen. Opvallend was wel dat het maladaptief eetgedrag ook op zijn beurt de angstgevoelens (als stressconstruct) kon verhogen.

Een tweede onderzochte leefstijlparameter was lichaamsbeweging. Zowel objectieve als gerapporteerde lichaamsbeweging was longitudinaal toegenomen bij hoge stress. Deze relatie was echter verschillend naargelang de leeftijd: negatieve emoties deden de lichaamsbeweging stijgen bij de jongere groep, terwijl een verminderde lichaamsbeweging werd veroorzaakt bij de oudere kinderen. Op sedentair gedrag (gerapporteerd en gemeten) werd geen longitudinaal effect van stress gevonden.

Een laatste onderzochte leefstijlparameter was slaap. Een korte slaapduur maar niet de lage slaapkwaliteit was cross-sectioneel geassocieerd met hoge stress (vooral negatieve emoties). Longitudinale relaties met slaap werden echter niet gevonden.

Alhoewel er geen direct longitudinaal effect van stress op overgewicht was, werd deze relatie wel versterkt of verzwakt door leefstijl en cortisol. Dit leidde tot positieve stress-overgewicht associaties bij kinderen met een hoog cortisolpatroon en een ongezonde leefstijl. Tevens werd een significante relatie gevonden in de omgekeerde oorzaak-gevolg richting: lichaamssamenstelling beïnvloedde het stressniveau daar kinderen met een hogere BMI of een hoger vetpercentage één jaar later meer negatieve emoties rapporteerden.

Discussie

De resultaten bevestigen dat **stress bij kinderen** een belangrijke determinant is voor hormonale en longitudinale leefstijlveranderingen (vooral een ongezonde voeding en eetgedrag) die dan eventueel later de ontwikkeling van overgewicht kunnen stimuleren. Er bestaan evenwel individuele verschillen (leeftijd en geslacht) in deze hormonale reacties en leefstijlreacties op stress. Deze verschillen zullen deels het risico op overgewicht bepalen daar cortisol en leefstijl de stress-overgewicht relatie beïnvloeden. Bijgevolg speelt zowel de cortisol- als de leefstijlhypothese een rol in de stress-overgewicht relatie. Langere opvolgstudies zullen echter nodig zijn om de verdere evolutie van het gewicht te traceren. Naarmate kinderen ouder worden, zal de rol van leefstijl in deze relatie wellicht in belang toenemen daar adolescenten meer inspraak krijgen in hun voedingskeuzes en mogelijk minder tijd hebben om fysiek actief te zijn. De stress-overgewicht relatie bleek verder bidirectioneel te zijn daar de lichaamssamenstelling en het eetgedrag ook een longitudinale invloed uitoefenden op een aantal stressaspecten.

Aangezien zowel stress als overgewicht in grote mate bijdragen tot de ziektecijfers, dienen **preventiestrategieën** uitgedacht te worden.

Strategieën bij het aanpakken van de stress-overgewicht relatie kunnen ontwikkeld worden op verschillende niveaus en rond verschillende aspecten. Essentiële aspecten zijn stress screening, stressmanagement, stressbehandeling, leefstijlinterventie en de behandeling van overgewicht. Stressmanagement kan focussen op probleemoplossende vaardigheden en emotionele regulatie terwijl ook op positieve aspecten kan gewerkt worden zoals sociale ondersteuning en zelfvertrouwen. Betreffende de leefstijl kan de omgeving best omgevormd worden tot een ‘activiteitsaanmoedigende, gezonde voedingszone’ die de gelegenheid tot emotioneel eten dient te minimaliseren en de kans tot lichaamsbeweging te maximaliseren. Aangezien de stress-overgewicht relaties bidirectioneel kunnen zijn, dient het gewicht van mensen met psychosociale symptomen opgevolgd te worden en dient ook de gemoedstoestand

van mensen met overgewicht opgevolgd te worden in het idee van een transdisciplinaire behandeling van overgewicht. Al deze initiatieven kunnen uitgevoerd worden op het niveau van het kind, de familie, de school of de gemeenschap. Wegens de grote focus van huidige initiatieven op adolescenten, zou de aandacht ook verschoven moeten worden naar de jongere groep van lagere schoolkinderen.

Uiteindelijk werden ook enkele suggesties gedaan voor **verder onderzoek** bij populaties met een hoog risico (kinderen met overgewicht of gezinnen met een lage sociale status), rond methodologische verfijningen, de rol van contextuele invloeden (bv de ouders), het integreren van een levensperspectief door ook perinatale stress te beschouwen, de focus te verbreden naar het metabool syndroom als uitkomstfactor en het verder doorgronden van onderliggende aspecten zoals de eetlustregulerende hormonen en de beloningsgevoeligheid.

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ANNEX

A) Coping questionnaires

Wat doe je wanneer je je zorgen maakt of van streek bent of problemen hebt?			
1) met iemand praten	nooit	soms	vaak
2) mezelf de schuld geven dat ik problemen heb	nooit	soms	vaak
3) kwaad worden / wenen	nooit	soms	vaak
4) in mijn kamer blijven	nooit	soms	vaak
5) denken wat ik doe als ik hetzelfde probleem heb	nooit	soms	vaak
6) iets snoepen of eten	nooit	soms	vaak
7) proberen aan iets anders te denken	nooit	soms	vaak
8) denken aan wat gebeurd is en hoe ik het kan oplossen	nooit	soms	vaak

B) Emotions questionnaire

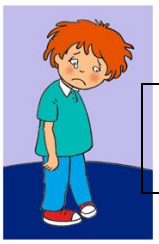
Hoe voel je je meestal (niet alleen vandaag)? Duid een cijfer aan tussen 0 (nee) en 10 (ja).

Blij

0 Nee	1	2	3	4	5 Een beetje	6	7	8	9	10 Ja

Boos

0 Nee	1	2	3	4	5 Een beetje	6	7	8	9	10 Ja



Verdrietig

0 Nee	1	2	3	4	5 Een beetje	6	7	8	9	10 Ja

Bang

0 Nee	1	2	3	4	5 Een beetje	6	7	8	9	10 Ja

DANKWOORD -

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Nathalie

CURRICULUM VITAE

Personalia

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Date of birth	18th September 1986
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Education

2009 - 2013	Doctoral schools Training Programme (conference skills, scientific writing skills, personal effectiveness, teaching assistant, communication skills, proposal writing, leadership foundation, introduction to clinical studies, hospital management, project management, 4 advanced statistic courses)
2009	COSPI intensive and interactive course ("Combating Obesity: Strategies for Prevention and Intervention"), Graz, Austria. 22th February – 8th March 2009.
2004 - 2009	Master Biomedical Sciences – option 'Nutrition' (summa cum laude) Ghent University
1998 - 2004	Secondary school: Latin – sciences Sint-Bernarduscollege Oudenaarde

Professional experience

2009 - present	PhD researcher at the Department of Public Health Ghent University
2009 - present	Ad hoc reviewer for scientific journals
2009 - 2013	Supervision of 14 master thesisses at the Department of Public Health Ghent University
2009 - 2010	Fieldwork organization and implementation of the IDEFICS study Department of Public Health, Ghent University
2009	Scientific interviewer for the KANS study Department of Family Medicine and Primary Health Care, Ghent University
2008-2009	Research for master thesis: 'Effect of liver cirrhosis on malnutrition in rats' Supervisor: Prof Dr. Van Vlierberghe Hans, Ghent University

A1 first authorship

1. Michels N, Sioen I, De Vriendt T, Huybrechts I, Vanaelst B, De Henauw S, 2011. Children's morning and evening salivary cortisol: pattern, instruction compliance and sampling confounders. *Hormone Research in Pediatrics,* 77: 27-35.

2. Michels N, 2012. Absence of Cortisol Awakening Response in Children and Salivary Cortisone as a Related, Promising Stress Marker. *Hormone Research in Pediatrics,* 77(2): 135-6.

3. Michels N, Sioen I, Huybrechts I, Bammann K, Vanaelst B, De Vriendt T, Iacoviello L, Konstabel K, Ahrens W, De Henauw S, 2012. Negative life events, emotions and psychological difficulties as determinants of salivary cortisol in Belgian primary school children. *Psychoneuroendocrinology,* 37(9): 1506-15.

4. Michels N, Vanaelst B, Stoppie E, Huybrechts I, Bammann K, De Henauw S, Sioen I, 2013. Parental and children's report of emotional problems and stressful life events: agreement, explanatory factors and event-emotion correlation. *Child and Adolescent Mental Health*, 18(3): 180-6.

5. Michels N, Huybrechts I, Bammann K, Lissner L, Moreno L, Peeters M, Sioen I, Vanaelst B, Vyncke K, De Henauw S, 2013. Caucasian children's fat mass: routine anthropometry versus air-displacement plethysmography. *British Journal of Nutrition*, 109 (8): 1528-37.

6. Michels N, Sioen I, Braet C, Eiben G, Hebestreit A, Huybrechts I, Vanaelst B, Vyncke K, De Henauw S, 2012. Stress, emotional eating behaviour and dietary patterns in children. *Appetite,* 59(3): 762-9.

7. Michels N, Clays E, De Buyzere M, Huybrechts I, Marild S, Vanaelst B, De Henauw S, Sioen I, 2013. Determinants and reference values of short-term heart rate variability in children. *European Journal of Applied Physiology*, 113(6): 1477-88.

8. Michels N, Sioen I, Braet C, Huybrechts I, Vanaelst B, Wolters M, De Henauw S, 2013. Relation between salivary cortisol as stress biomarker and dietary pattern in children. *Psychoneuroendocrinology,* 38: 1512-20.

9. Vanaelst B*, Michels N*, Clays E, Herrmann D, Huybrechts I, Sioen I, Vyncke K, De Henauw S, 2013. The relationship between childhood stress and body composition, and the role of stress-related lifestyle factors - cross-sectional findings from the baseline ChiBS survey. *International Journal of Behavioral Medicine,* Epub.

10. Michels N, Clays E, De Buyzere M, Vanaelst B, De Henauw S, Sioen I, 2013. Children's sleep and the autonomic function: low sleep quality has an impact on heart rate variability independently of stress. *Sleep*, 36(12): 1939-46.

11. Michels N, Sioen I, Clays E, De Buyzere M, Ahrens W, Huybrechts I, Vanaelst B, De Henauw S, 2013. Children's heart rate variability as stress indicator: association with reported stress and cortisol. *Biological Psychology*, 94(2): 433-40.

A1 co-authorship

1. Stomfai S, Ahrens W, Bammann K, Kovács E, Marild S, Michels N, Moreno LA, Pohlabeln H, Siani A, Tornaritis M, Veidebaum T, Molnár D, 2011. Intra - and interobserver reliability in anthropometric measurements. *International Journal of Obesity*, 35(S1): S45-51.

2. Sioen I, Mouratidou T, Kaufman JM, Bammann K, Michels N, Pigeot I, Vanaelst B, Vyncke K, De Henauw S, 2011. Determinants of vitamin D status in young children: results from the Belgian arm of the IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) Study. *Public Health Nutrition,* 8: 1-7.

3. Vanaelst B, De Vriendt T, Ahrens W, Bamman K, Barba G, Chadjigeorgiou C, Konstabel K, Lissner L, Michels N, Molnar D, Moreno L, Reisch L, Sioen I, De Henauw S, 2012. Prevalence of psychosomatic problems in European school-aged children and their association with childhood adversities. *European Child & Adolescent Psychiatry*, 21(5): 253-65.

4. Vyncke KE, Libuda L, De Vriendt T, Moreno LA, Van Winckel M, Manios Y, Gottrand F, Molnar D, Vanaelst B, Sjöström M, González-Gross M, Censi L, Widhalm K, Michels N, Gilbert CC, Xatzis C, Cuenca García M, Pérez de Heredia F, De Henauw S, Huybrechts I, 2012. Dietary fatty acid intake, its food sources and determinants in European adolescents. The HELENA study. *British journal of nutrition,*108(12): 2261-73.

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6. Van den Bussche K, Michels N, Gracia-Marco L, Herrmann D, Eiben G, De Henauw S, Sioen I, 2012. Influence of birth weight on calcaneal bone stiffness in Belgian pre-adolescent children. *Calcified Tissue International,* 91(4): 267-75.

7. Börnhorst C, Huybrechts I, Ahrens W, Eiben G, Michels N, Pala V, Molnár D, Russo P, Barba G, Bel-Serrat S, Moreno LA, Papoutsou S, Veidebaum T, Loit HM, Lissner L, Pigeot I, 2013. Prevalence and determinants of misreporting among European children in proxy-reported 24-hour dietary recalls. *British Journal of Nutrition,* 109(7): 1257-65.

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9. Vanaelst B, Huybrechts I, Michels N, Flórez MR, Balcaen L, Resano M, Vanhaecke F, Taes Y, Bammann K, Bel-Serrat S, De Henauw S, 2013. Hair minerals and metabolic

health in Belgian elementary-school children. *Biological Trace Element Research*, 151(3): 335-43.

10. Bel S, Michels N, De Vriendt T, Patterson E, Cuenca-Garcia M, Diethelm K, Gutin B, Grammatikaki E, Manios Y, Leclercq C, Ortega FB, Moreno LA, Gottrand, Widhalm K, Kafatos A, Garaulet M, Molnar D, Kaufman JM, Gilbert C, Lena H, Sjöström M, Kersting M, Gonzalez-Gross M, De Henauw S, 2013. Association between self-reported sleep duration and dietary intake in European adolescents. *British Journal of Nutrition*, 110(5): 49-59.

11. Vanaelst B, Michels N, De Vriendt T, Huybrechts I, Vyncke K, Sioen I, Bammann K, Rivet N, Raul JS, Molnar D, De Henauw S, 2013. Cortisone in hair of elementary school girls and its relationship with childhood stress. *European Journal of Pediatrics*, 172(6): 843-6.

12. Vanaelst B, Michels N, Huybrechts I, Clays E, Florez MR, Balcaen L, Resano M, Aramendia M, Vanhaecke F, Rivet N, Raul JS, Lanfer A, De Henauw S, 2013. Cross-sectional relationship between chronic stress and mineral concentrations in hair of elementary school girls. *Biological trace element research*, 153(1-3): 41-9.

13. Vandevijvere S, Michels N, Verstraete S, Ferrari M, Kersting M, Gonzalez-Gross M, Moreno L, Mouratidou T, Grammatikaki E, Valtueña J, Cuenca Garcia M, Meirhaeghe A, Stevens K, Dallongeville J, De Henauw S, Huybrechts I, 2013. Intake and dietary sources of haem and non-haem iron among European Adolescents and its association with iron status and different lifestyle and socio-economic factors. *European Journal of Clinical Nutrition*, 67(7): 765-72.

14. Hense S, Pohlabeln H, Michels N, Marild S, Lissner L, Kovacs E, Moreno L, Hadjigeorgiou C, Veidebaum T, Iacovello L, Pitsiladis Y, Reisch L, Siani A, Ahrens W. Determinants of attrition to follow up in a multi-centre cohort study in children – results from the IDEFICS Study. *Epidemiology Research International*, In press.

A2 publications

1. Michels N, Vanaelst B, Vyncke K, Sioen I, Huybrechts I, De Vriendt T, De Henauw S, 2012. Children's Body composition and Stress – the ChiBS study: aims, design, methods, population and participation characteristics. *Archives of Public Health*, 70(17).

2. Vanaelst B, Huybrechts I, De Bourdeaudhuij I, Bammann K, Chadjigeorgiou C, Eiben G, Konstabel K, Michels N, Molnar D, Moreno L, Pigeot I, Reisch L, Siani A, Vyncke K, De Henauw S. Prevalence of negative life events and chronic adversities in European school-aged children: results of the IDEFICS study. *Archives of Public Health*, 70(26).

A4 publications

1. Michels N, Vanaelst B, Vyncke K, 2010. Symposium: 'Borstvoeding in België: van kennis tot praktijk'. *Voeding Nu* (January).

2. Michels N, Vanaelst B, De Vriendt T, 2012. Stress als determinant voor overgewicht. *Voeding Nu* (January).

3. Michels N, Vanaelst B, Vyncke K, Sioen I, Huybrechts I, De Vriendt T, De Henauw S, 2012. Relatie tussen stress en lichaamssamenstelling bij kinderen: design en eerste resultaten van de ChiBS studie. *Tijdschrift voor voeding en diëtetiek*, 18(5): 8-12.

Scientific reports

Michels N, Claessens M, Huybrechts I, De Henauw S. Breakfast cereal consumption in European adolescents: descriptive data and differences in lifestyle, anthropometry and biomarkers. Results of the HELENA study. Report, Ghent: Department of Public Health, May 2013.

Presented congress abstracts

1. BNS (Belgian nutrition society) annual conference: 'Lipids in nutrition', Brussels, 23th April 2010 (1 poster presentation)

2. IDEFICS symposium: 'Child health in Europe: towards a better understanding of obesity', Zaragoza, 8th-9th November 2010 (3 poster presentations)

3. 20th ECOG congress: 'Taking childhood obesity off the menu', Brussels, 17th-20th November 2010 (1 poster presentation)

4. EASO Bjorntorp symposium: 'Neuroendocrine influence on the metabolic syndrome', Gothenburg, Sweden, 29th June – 1st July 2011 (1 poster presentation)

5. ISPNE Annual conference: 'Circadian rhythms', Berlin, Germany, 4-6th August 2011 (1 poster presentation)

6. FENS European nutrition conference, Madrid, Spain, 26th-29th October 2011 (1 poster presentation)

7. American Psychosomatic Society Annual Meeting, Athens, Greece, 14-17th March 2012 (2 poster presentations)

8. BNS annual conference 'Behaviour and nutrition', Brussels, Belgium, 20th April 2012 (1 oral, 1 poster presentation)

9. ISPNE annual conference: 'Molecular and hormonal effects of traumatic stress', New York, USA, 10-14th September 2012 (2 oral, 1 poster presentation)

10. Meeting at the Division of Endocrinology, Diabetes and Metabolism in the CHUV, Lausanne, Switzerland, 9th October 2012 (invited speaker by Puder Jardena)

11. Annual meeting of the European Childhood Obesity Group, Mallorca, 17-19th October 2012 (1 oral presentation)

12. American Psychosomatic Society Annual Meeting, Miami, 13-16th March 2013 (3 poster presentations)
13. What basic sciences for child psychiatry: Mind, Brain and Emotions, Rome, Italy, 16-17th May 2013 (invited oral presentation)
14. 'International society of behavioral nutrition and physical activity' annual meeting, Ghent, Belgium, 22-25th May 2013 (oral presentation)
15. ISPNE annual conference: 'Stress, rhythm and blues', Leiden, The Netherlands, 20-22th August 2013 (1 oral, 1 poster presentation)
16. World congress on psychosomatic medicine, Lisbon, Portugal, 12-14th September 2013 (1 oral)
17. Voedings- en Gezondheidscongres, Brussels, Belgium, 22-23th November 2013 (invited oral presentation)

Awards

1. Travel award for the ISPNE Eating and Addiction Conference 2012 in New York
2. Louis Bonduelle Research award 2012