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Acute Stress and Traumatic Stress in Type 2 Diabetes Mellitus

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List of Abbreviations

ACTH	adrenocorticotropic hormone
ANS	autonomic nervous system
CAN	cardiac autonomic neuropathy
CAR	cortisol awakening response
CRH	corticotropin-releasing hormone
CTQ	childhood trauma questionnaire
GR	glucocorticoid receptor
HF HRV	high frequency heart rate variability
HPA axis	hypothalamic–pituitary–adrenal axis
HR	heart rate
HRV	heart rate variability
LF HRV	high frequency heart rate variability
MR	mineralocorticoid receptor
PNS	parasympathetic nervous system
PVN	paraventricular nucleus
RSA	respiratory sinus arrhythmia
SA node	sinoatrial node
SNS	sympathetic nervous system
TSST	trier social stress test
VAS	visual analogue rating scale

1 Introduction

Type 2 diabetes, a chronic illness defined by insulin resistance and -deficiency caused by pancreatic beta cell dysfunction (Chatterjee et al. 2017), and its complications are a growing global health concern. Currently, more than 6% of people worldwide (Khan et al. 2020) and more than 8% of the German population suffer from type 2 diabetes (Tönnies et al. 2019). In spite of extensive research and efforts to advance treatment of type 2 diabetes, prevalence rates and diabetes-associated deaths are still on the rise (Khan et al. 2020; Tönnies et al. 2019). In an effort to gain a more comprehensive understanding of the disease, diabetes research has expanded into the field of psychological factors with a focus on chronic and traumatic psychological stress (Pouwer et al. 2010). Stress has thus become a widely researched risk factor for disease development and progress (Hackett and Steptoe 2017; Kelly and Ismail 2015). However, there are at least two areas concerning the role of stress in type 2 diabetes that are of special interest from a psychosomatic point of view and have not yet been investigated.

In type 2 diabetes research, psychological stress had been hypothesized to act as both, a risk factor for developing type 2 diabetes or diabetic complications as well as a consequence of type 2 diabetes and its complications. Research has shown the close-knit relationship between the physiological stress system, i.e. the hypothalamus pituitary adrenal axis (HPA axis) and the autonomic nervous system (ANS), and the physiological systems directly involved in the pathology of type 2 diabetes i.e. the metabolic system (Rabasa and Dickson 2016) and the immune system (Hameed et al. 2015; Yaribeygi et al. 2017). Investigating the physiological stress response system in type 2 diabetes could therefore be pivotal in understanding the relationship between stress and type 2 diabetes as well as the disease as a whole. However, only limited research has been done into the stress response in type 2 diabetes and most research has been focused on the HPA axis' response to stress (Faulenbach et al. 2012; Steptoe et al. 2014) while the stress response of the ANS in type 2 diabetes patients has not been studied comprehensively. As decreased function of the ANS and especially autonomic control over the cardiovascular system has been shown to precede (Lee et al. 2020) as well as result from type 2 diabetes (Benichou et al. 2018) the stress response of the ANS is likely to be a key factor in the relationship between type 2 diabetes and stress. The first area of interest of the present study will thus be the cardiac autonomic stress response in type 2 diabetes.

Among the most formative kinds of psychological stress is the experience of childhood maltreatment. Childhood maltreatment has been shown to increase the risk for many chronic diseases in adulthood (Felitti et al. 1998; Norman et al. 2012) and recently, longitudinal studies

have demonstrated a link between type 2 diabetes and childhood maltreatment (Huffhines et al. 2016). Studies distinguishing different forms of childhood maltreatment have hereby found the strongest association between type 2 diabetes and childhood physical and emotional neglect (Huang et al. 2015). However, possible mechanisms that link childhood maltreatment to type 2 diabetes are still unclear. As the stress response system is particularly susceptible to experiences of stress during its development in childhood, childhood neglect can have lasting effects on physiology. Moreover, alterations of the stress response system, as they have been described in type 2 diabetes, are a common finding in samples with a background of childhood neglect (Reilly and Gunnar 2019). An altered stress response is thus likely part of the mechanism connecting childhood neglect and type 2 diabetes. Nevertheless, the effects of childhood neglect on the stress response of type 2 diabetes patients remain to be studied. The second area of interest of this study will therefore concern associations between childhood neglect and the stress response in type 2 diabetes patients.

2 Background

2.1 Stress in Type 2 Diabetes

Within the past decades, type 2 diabetes has frequently been connected to the experience of psychological stress (Falco et al. 2015; Lloyd et al. 2005; Wales 1995). Research has shown that patients suffering from type 2 diabetes show increased levels of perceived stress as well as a higher risk for stress-related psychological disorders such as depression and anxiety (Bo et al. 2019; Lloyd et al. 2018). On the one hand, type 2 diabetes can hereby be understood as a stressor in itself. Suffering from a lifestyle-associated, chronic disease with treatment demanding high levels of self-management and social stigma attached to the disease can be a cause of chronic stress (Skinner et al. 2020; Tareen and Tareen 2017). Patients also commonly experience fear of acute complications such as hypoglycemic events (Sakane et al. 2015) or the development of complications (Kuniss et al. 2019).

On the other hand, it has also been demonstrated that chronic stress can act as a risk factor for the development of type 2 diabetes. In a study Madhu et al. (2019) assessed perceived stress in a sample of 500 participants with newly detected type 2 diabetes before they were told their diagnosis and 500 matched, healthy controls participants. They found significantly higher levels of stress (OR=1.2; CI: 1.09–1.24) in participants with type 2 diabetes. Nyberg et al. (2014) assessed job-strain in 124,808 adults and type 2 diabetes incidence at follow-up 10 years later. They found job-strain to be an independent predictor for the development of type 2 diabetes (HR: 1.15; CI: 1.06-1.25). Stress has also been implicated as a factor in the development of chronic diabetic complications. In a sample including 4,090 type 2 diabetes patients Cummings et al. (2016) report an increased incidence of stroke (HR: 1.57, CI:1.05, 2.33) for patients reporting elevated levels of stress or depression. A study in 1533 type 2 diabetes patients by Dalsgaard et al. (2014) found a 1.8 increase in mortality rates and a 1.7 increase in the risk for cardiovascular events in patients suffering from psychological distress. Of course, psychological stress needs to be understood as one of many risk factors (Bi et al. 2012). But while other risk factors may have a stronger impact, stress is in many cases a modifiable risk factor (Hackett and Steptoe 2017) and is therefore of major importance.

Consequently, psychological stress has become a target for treatment and interventions such as mindfulness based stress reduction have shown positive effects in type 2 diabetes patients (for a review see Mason et al. 2018) and have been shown to be effective in preventing the development of diabetic complications (Hartmann et al. 2012). High levels of stress have repeatedly been linked to unhealthy lifestyle habits such as physical inactivity or smoking (Rod et al. 2009)

as well as poor treatment adherence in type 2 diabetes patients (Gonzalez et al. 2015). These effects of stress are thus a likely mediator in the relationship of stress and the development and course of type 2 diabetes. However, there is evidence that the association between stress and type 2 diabetes persists when lifestyle-associated factors and health behaviors are controlled for (Eriksson et al. 2008; Nyberg et al. 2014), implicating additional mechanisms within the physiological stress response system mediating the relationship.

2.2 The Physiological Stress Response System in Type 2 Diabetes

The central actors of the physiological stress response system are two distinct but interrelated systems: the HPA axis and the ANS. Both systems have been shown to be involved in the pathogenesis and pathology of type 2 diabetes and its complications. In the following, the physiology of ANS and the HPA axis, common methods to assess their activity, their significance in the field of psychosocial influence factors on health and disease as well as their role in type 2 diabetes pathology will be described.

2.2.1 The Autonomic Nervous System

The ANS consists of two divisions, the sympathetic and the parasympathetic nervous system (SNS and PNS). The SNS and the PNS share regulatory influence on most tissues and organ systems in the body and generally have opposing effects on target organs. Both systems are tonically active, meaning they always provide a certain degree of nervous input and adaptive modulation of tissue activity takes place via an increase or decrease in frequency. A complex interplay of activation and inhibition of the PNS and SNS ensures efficient adaption to changes in internal or external conditions with the PNS dominating during resting- and the SNS during active states. The divisions of the ANS have often been described to act in an antagonistic fashion with activation of the PNS resulting in inhibition of the SNS, however they have also been shown to act independently or even coactively. Stressful conditions generally lead to parasympathetic withdrawal, sympathetic activation, or both, preparing the organism for activity (Ernst 2011; McCorry 2007).

Sympathetic activation stimulates the production of epinephrine and norepinephrine in the adrenal medulla. Epinephrine and norepinephrine then bind on receptors within target organs and can increase cardiac output, cause vasodilatation in muscles, bronchodilation and stimulate glycogenolysis. The main neurotransmitter facilitating parasympathetic activity is acetylcholine. Acetylcholine binds on cholinergic receptors and decreases cardiac output, stimulates digestion

and insulin secretion from the pancreas, lowering blood glucose levels. The longest and most important nerve of the PNS is the vagus nerve. It innervates the thorax and the abdomen, making it a central component in autonomic regulation of cardiac activity (Battipaglia and Lanza 2015; Ernst 2011).

2.2.1.1 Heart Rate and Heart Rate Variability

Both divisions of the ANS are modulators of cardiac activity. Norepinephrine as well as acetylcholine bind to receptors on the sinoatrial (SA) node, a group of so-called pacemaker cells of the heart. These cells produce an intrinsic heart beat without autonomic or hormonal input. This intrinsic heartbeat (approximately 100 to 115 bpm in healthy adults) is constantly modified through tonic and phasic SNS and PNS influence. Binding of norepinephrine leads to an increased excitability of pacemaker cells, a decrease in heart period and thus to an acceleration of the heartbeat. Vagal release of acetylcholine on the other hand slows diastolic depolarization and leads to an increase in heart period and a decrease in heart rate (HR; de Geus et al. 2019). HR changes in response to a (mental or physical) stressor are therefore closely linked to the interplay of the PNS and the SNS and the quality of autonomic control of the heartbeat. HR reactivity as well as recovery (i.e. the return to baseline levels after stressor cessation) have become well-established indicators of cardiac autonomic control and flexibility. While the magnitude of the increase in HR in response to a stressor is related to the rapid effects of inhibition of vagal activity and an activation of sympathetic impulses, HR recovery is linked to effects of vagal reactivation as well as sympathetic withdrawal (Okutucu et al. 2011). Attenuated HR recovery has been associated with an increase in all-cause mortality (Qiu et al. 2017) and symptoms of psychopathology (Gordon et al. 2012). HR hypo-reactivity is a common finding in patients with major depression (Schiweck et al. 2019) and is associated with adverse health behaviors (Ginty et al. 2016). These findings highlight the close relationship between autonomic flexibility and mental and physical health.

A second indicator of autonomic activity is heart rate variability (HRV; Ernst 2017; Ziemssen and Siepmann 2019). Through the constant autonomic and hormonal modulatory influence on cardiac activity, the time intervals between heart beats fluctuate. The magnitude and quality of these fluctuations over time reflect autonomic balance, autonomic flexibility and the ability of the organism to cope with changing environmental demands. Depending on the duration of the measurement interval, different frequencies of fluctuation can be assessed. While 24-h recordings of HRV can for example depict fluctuation in cardiac activity caused by the circadian

rhythm, short-term recordings (e.g. 5 minutes) will primarily assess high frequency fluctuation (0.15-0.4 Hz). In terms of the ANS, short recordings and higher frequency HRV are generally understood to predominantly reflect vagal modulatory influences, as those are faster (<1s) in affecting heart rhythm than sympathetic (>5s) influences (Shaffer and Ginsberg 2017). An important source of high frequency HRV is respiratory sinus arrhythmia (RSA). RSA describes the shortening of the R-R interval during inspiration and lengthening of the R-R interval during expiration. As this mechanism of cardiorespiratory coupling is mainly mediated through vagal activity, RSA can be understood as an indicator of tonic vagal activity. Both, HR and HRV are often measured to assess the physiological response to stress.

In healthy samples, HR has been reported to raise during stressor anticipation, peak during acute stress and return to pre-stress levels within approximately 5 min after stressors cessation (Allen et al. 2014). For HRV, studies report an opposing pattern, with measures of overall HRV and parasympathetic activity decreasing during stressor anticipation and during acute stress through the shift towards sympathetic dominance (Castaldo et al. 2015). After stressor cessation, HRV will normally recover again within approximately 5 minutes. In healthy subjects, studies have additionally reported an increase in vagal modulatory activity compared to baseline levels shortly after stress, due to so called vagal rebound (Mezzacappa et al. 2001). The magnitude of the cardiac autonomic response to stress strongly varies depending on the nature and context of the stressor as well as on individual factors. Older age groups for example show attenuated HR and HRV responses to stress (Strahler et al. 2010), and better physical fitness has been linked to a decreased area under the curve for HR (Wyss et al. 2016). Importantly in the context of type 2 diabetes, body mass index (BMI) has been shown to be inversely related to HRV (Koenig et al. 2014).

Loss of dynamic variability and decreased autonomic flexibility have been associated with many adverse health outcomes including obesity, hypertension, and type 2 diabetes (Masi et al. 2007; Wulsin et al. 2018). Decreased autonomic flexibility has hereby been discussed as a cause as well as a consequence of chronic disease. Additionally, autonomic flexibility has been a focus of psychophysiological research. In this context, decreased autonomic flexibility and vagal tone have been linked to an increased vulnerability to the effects of stress (Weber et al. 2010) and a higher risk of developing stress-related conditions such as sleep disturbances, depression (Da Estrela et al. 2021) and burn-out (Wekenborg et al. 2019).

2.2.1.2 The ANS in Type 2 Diabetes

Type 2 diabetes patients frequently show an increased resting HR as well as a decreased HRV (Vinik and Ziegler 2007), indicating reduced autonomic modulation of the cardiovascular system. These changes in autonomic function are commonly attributed to decreased vagal modulation and a shift towards sympathetic dominance (Vinik et al. 2011). However, a meta-analysis of 25 studies on autonomic function in type 2 diabetes patients reported decreased HRV across almost all HRV parameters implying reduced parasympathetic as well as sympathetic activity (Benichou et al. 2018). The pattern auf autonomic dysfunction in type 2 diabetes presumably depends on illness duration and progression, and parasympathetic activity might be affected before sympathetic activity (Goit et al. 2012).

In diabetes research, impaired autonomic control over cardiovascular activity is predominantly understood as a consequence of cardiac autonomic neuropathy (CAN), a common complication of type 2 diabetes with prevalence rates of up to 75% (Agashe and Petak 2018). CAN is a major cardiovascular risk factor and strongly associated with mortality (Vinik et al. 2018). The pathogenic process leading to CAN is not yet fully understood. Hyperglycemia is assumed to be a major factor as increased blood glucose levels can lead to injury and death of neural cells and damage DNA via several known pathways such as the formation of reactive oxygen species and advanced glycation end products (Fisher and Tahrani 2017; Sharma et al. 2020). On the other hand, longitudinal studies indicate that decreased parasympathetic modulation and autonomic imbalance often precedes type 2 diabetes and may even play a role in pathogenesis (Hoshi et al. 2019; Lee et al. 2020). The ANS is a central actor in blood glucose regulation. Autonomic fibers innervate the pancreas and can stimulate as well as inhibit insulin secretion. They influence glucose uptake in skeletal muscle, lipolysis in adipose tissue and gluconeogenesis and glycogenolysis in the liver (Lindmark et al. 2005). Autonomic imbalance can therefore have deleterious consequences for metabolic control and is related to many risk factors of type 2 diabetes such as obesity, metabolic syndrome and insulin resistance (Wulsin et al. 2015).

The ANS thus shows impaired function among type 2 diabetes patients, is involved in type 2 diabetes pathology and may even play a role in pathogenesis. An altered autonomic stress response in type 2 diabetes patients is therefore likely. However, literature on the autonomic and cardiac autonomic stress response in type 2 diabetes patients is sparse. To date, only one study by Steptoe et al. (2014) has assessed the cardiac autonomic response to a psychological stressor in type 2 diabetes patients. The authors report a blunted HR response with an increased baseline HR and an attenuated HR recovery, a pattern indicative of decreased autonomic flexibility.

Importantly however, they used a comparatively weak, cognitive stress paradigm, did not assess HRV, and excluded people with signs of CAN, thus excluding a significant subgroup of type 2 diabetes patients. One major aim of the present study was therefore the investigation of the cardiac autonomic stress response in type 2 diabetes compared to healthy controls. Furthermore, this study aimed at examining the role of diabetic complications in this context by additionally comparing the subgroup of type 2 diabetes patients with diabetic complications to healthy controls.

2.2.2 The HPA Axis

The HPA axis can be understood as a complex physiological system of activating influences and inhibiting feedback mechanisms between three main components: the hypothalamus, the pituitary and the adrenal glands. The physiological changes caused by the activation of the HPA axis through a stressor are slower than those caused by the ANS and peak approximately 20 minutes after the occurrence of a stressor (Gunnar and Quevedo 2007). The stress response of the HPA axis is initiated by the release of corticotrophin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus. CRH stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland into the blood circulation. ACTH then travels to the adrenal cortex, triggering the synthesis of cortisol (Holsboer and Ising 2010). The release of cortisol from the adrenal cortex occurs in pulses and during a stress reaction the amplitude and frequency of these pulses increase. Cortisol binds on two types of receptors: the mineralocorticoid receptors (MRs) and the glucocorticoid receptors (GRs). MRs bind cortisol with a high affinity and are widely saturated during regular functioning. GRs bind cortisol with a lower affinity and are therefore able to retain sensitivity during phases of high hormonal levels. GRs occur in almost all tissues of the body including the central nervous system and in all precedent parts of the HPA axis. Through GR-binding in the PVN and the pituitary, corticosteroids inhibit the CRH and ACTH synthesis and thus downregulate HPA axis activity (Pariante and Lightman 2008). This feedback mechanism is necessary to restore baseline conditions after a strong secretory period and to ensure the responsiveness of the HPA axis to subsequent stressors (Holsboer and Ising 2010). The HPA axis response to stress depends on the nature of the stressor with social threat being among the strongest triggers (Giles et al. 2014). Hormonal output of the HPA axis varies strongly between individuals, with men on average showing stronger ACTH and cortisol responses then women and the HPA axis response to stress generally declining with age (Allen et al. 2017; Kudielka et al. 2004).

The effects of cortisol are widespread and affect almost all organ systems of the body. Within the stress response, cortisol affects the cardiovascular system by enhancing the aforementioned effects of catecholamines (increased cardiac output). High cortisol also increases blood glucose levels and mobilizes stored energy by acting on the liver, pancreas and adipose tissue. In the liver, cortisol increases gluconeogenesis while decreasing insulin release from pancreatic beta cells. In adipose tissue cortisol stimulates lipolysis, thus releasing glycerol and free fatty acids into the blood stream (Sapolsky et al. 2000; Thau and Sharma 2019).

Independent from the stress response, cortisol is secreted from the adrenal cortex throughout the day in a distinct diurnal rhythm. In healthy individuals, cortisol levels are lowest (<50 nmol/l) roughly two hours after sleep onset, then slowly start increasing to their highest levels (up to 399 nmol/l) in the morning after which they slowly degrade throughout the day. Apart from these periodic de- and increases of cortisol levels, independent peaks occur at meal intake and directly after awakening. The peak shortly after awakening is known as the cortisol awakening response (CAR; Selmaoui and Touitou 2003).

The activity of the HPA axis is commonly assessed by measuring cortisol concentration in blood, saliva or urine. Blood plasma cortisol levels provide a measurement of total cortisol while saliva will only reflect levels of unbound cortisol. Measuring plasma ACTH levels can provide additional information on the inner dynamics of HPA axis functioning. Depending on the research question, unstimulated (basal) HPA axis activity or HPA axis reactivity to a physical or psychological stressor can be measured.

HPA axis involvement has been described in a multitude of diseases and chronic conditions such as inflammatory and autoimmune diseases (Silverman and Sternberg 2012), metabolic disease and obesity (Incollingo Rodriguez et al. 2015), sleeping disorders (Asarnow 2020), depression (Menke 2019), post-traumatic stress disorder (Speer et al. 2019) and other mental disorders. Pathological HPA axis function characterized by hyper- or hypo-responsiveness, blunted diurnal cortisol curves or increased basal cortisol can result from physiological changes at any part of the HPA axis such as altered GR receptor density or sensitivity and changes in the HPA axis feedback mechanism (Karin et al. 2020). Those changes can occur as a form of adaption to intense, repeated or prolonged stimulation of the HPA axis as has been reported in the context of chronic stress (Miller et al. 2007) or trauma (Renée Klaassens, 2010; Speer et al. 2019). This pathway is often employed to explain the interaction of psychological and physiological factors in health and disease and is part of the reason the HPA axis has become a focus of psychosomatic research.

2.2.2.1 The HPA Axis in Type 2 Diabetes

HPA axis dysregulation among type 2 diabetes patients has been demonstrated within multiple paradigms, at times showing contradictory results regarding the specific pattern of dysregulation. Nevertheless, the most commonly described findings will be summarized shortly in the following. When compared to healthy controls, type 2 diabetes patients have been shown to exhibit a flattened diurnal cortisol curve with an attenuated CAR (Bruehl et al. 2009; Lederbogen et al. 2011) and increased evening cortisol levels (Siddiqui et al. 2015). The Dexamethasone suppression test often suggests a decreased feedback sensitivity in type 2 diabetes patients with higher cortisol levels after dexamethasone intake than healthy controls (Bruehl et al. 2007; Chiodini et al. 2005). HPA axis dysregulation and increased cortisol levels in type 2 diabetes patients have also been associated with measures of metabolic disturbance (Oltmanns et al. 2006) and a higher risk to develop diabetic complications (Reynolds et al. 2010).

Similar to the research on cardiac autonomic control, HPA axis dysfunction has been hypothesized to be among the causes as well as the consequences of type 2 diabetes. High blood glucose levels (Zänkert et al. 2020) as well as high glucose variability (George et al. 2014) have been shown to increase HPA axis activity, with chronic states of metabolic dysregulation leading to a change in HPA axis functioning. In a recent review however, Joseph and Golden (2017) proposed a model based on longitudinal data of the Whitehall II study in which chronic stress leads to a dysregulation of the physiological stress system and subclinical hypercortisolism which then facilitates the development of diabetes. As implied earlier, the HPA axis is involved in many metabolic processes and a dysregulation of the HPA axis can have detrimental effects on glucose control and increase type 2 diabetes risk, by causing beta-cell dysfunction, reducing insulin sensitivity (Di Dalmazi et al. 2012), activating lipolysis and promoting central obesity (Anagnostis et al. 2009). In addition, cortisol binds to receptors in immune cells and can affect the immune response. A prolonged state of HPA axis dysregulation can therefore promote a chronic low-grade inflammatory state (DeSantis et al. 2012) as it has been found in the pathogenesis of type 2 diabetes (Donath and Shoelson 2011).

The HPA axis response to acute stress in type 2 diabetes patients has been described in the aforementioned study by Steptoe et al. (2014). The authors reported increased baseline cortisol levels and a subsequently blunted saliva cortisol response to psychosocial stress in type 2 diabetes patients compared to healthy controls, showing that the altered functioning of the HPA axis in type 2 diabetes patients also affects the response to acute psychosocial stress.

Interestingly, Steptoe et al. (2014) report no difference in the subjective stress response between type 2 diabetes patients and healthy controls. Thus, describing a pattern of divergence with the alteration of the physiological stress response not being reflected in patients subjective experience. This result may be understood within the context of the research on alexithymia in type 2 diabetes. As a recent review by Martino et al. (2020) showed, rates of alexithymia are increased among type 2 diabetes patients and range between 25% to 50%. A reduced ability to identify and describe emotional states could explain why differences in the physiological stress response are not reflected in patients' subjective experience. On the other hand, Steptoe et al. (2014) only reported the results of a logistic regression analysis (adjusted odds) and no correlation between the physiological and the psychological stress response. An association between the physiological and the psychological stress response can consequently not be ruled out entirely.

2.3 Childhood Maltreatment, Childhood Neglect and Type 2 Diabetes

Among the most formative experiences of psychological stress is the chronic or repetitive exposure to maltreatment and deprivation in childhood. Childhood neglect and maltreatment have been shown to permanently affect the stress response as well as the immune and metabolic system (Berens et al. 2017; Miller et al. 2011) and increase the risk of many chronic diseases in adulthood including type 2 diabetes (Basu et al. 2017; Norman et al. 2012b). The literature on the association of type 2 diabetes with childhood maltreatment has grown substantially within the last years (Huffhines et al. 2016). Large, cross-sectional (Duncan et al. 2015; Rich-Edwards et al. 2010; Shields et al. 2016) as well as prospective studies (Lown et al. 2019; Thomas et al. 2008; Widom et al. 2012) have reported an increased risk for type 2 diabetes in samples with a background of childhood maltreatment.

Studies have also emphasized the importance of considering the severity and type of maltreatment (Rich-Edwards et al. 2010; Shields et al. 2016). A comprehensive review and meta-analysis by Huang et al. (2015) reported the strongest association with type 2 diabetes for experiences of physical and emotional neglect. This finding is particularly noteworthy as the consequences of childhood neglect are understudied compared to those of other forms of maltreatment (McSherry, 2007). At the same time, childhood neglect is the most common form of childhood maltreatment: a study by Witt et al. (2017) found that up to 9% of a representative German sample reported severe childhood neglect. Importantly, prevalence rates were found to steeply rise with age. In the age group of 50 to 59, 23% reported severe physical and 18% reported severe emotional neglect. In the age group of people older than 70, 46% reported

physical neglect. Childhood physical neglect is hereby defined as a failure of the caregiver to provide shelter, supervision, medical care, clothing or food. Emotional neglect on the other hand describes the inability of the caregiver to meet a child's emotional and developmental needs. Both forms of neglect are often chronic and co-occur with other forms of maltreatment (Reilly and Gunnar, 2019).

Childhood neglect can gravely affect a child's psychological and physiological development. Young children depend on their caregiver's attention for survival and experiencing neglect and deprivation signals to the child's organism that their environment is not safe and can cause extreme forms of stress (Reilly and Gunnar, 2019). Especially in the early stages of emotional and cognitive development the HPA axis (Tarullo and Gunnar, 2006) as well as the ANS (Quigley and Moore, 2018) go through sensitive periods in which these systems are especially impressionable. A caregiver's task is to co-regulate the infant's emotional reactions and act as so-called social buffer for the stress response system. Secure attachments with attentive caregivers are therefore crucially important (Adam et al. 2007; Gunnar and Donzella 2002). In the case of a neglected child, the stress response system is thus in a state of repeated or chronic activation. Employing the allostatic load model (Wen, 1998) over time, the function of the stress response system will be impaired and may become hyperactive, or through the process of desensitization, hypoactive.

2.3.1 Childhood Neglect and the Stress Response System

Children who are being raised by depressed or withdrawn mothers as well as institutionalized orphans already show patterns of HPA axis dysregulation at a very early age. While the results are generally mixed, studies commonly report increased basal cortisol levels (Bugental et al. 2003; Fries et al. 2008; Gunnar, et al. 2001) as well as attenuated diurnal cortisol slopes (Koss et al. 2014) and a blunted cortisol response to stress (Hostinar et al. 2015). This pattern of HPA axis dysregulation persists throughout adolescence (Bick et al. 2015; Bouma et al. 2011) and adulthood. Several studies (Kumsta et al. 2017; Power et al. 2012; van der Vegt et al. 2009) have shown attenuated or even absent cortisol awakening responses and flatted diurnal slopes in adult adoptees who experienced deprivation in early childhood. Literature focusing specifically on the effects of childhood neglect (rather than using a composite measure of general childhood adversity) on the HPA axis stress response is limited, however, mostly indicates an attenuated cortisol response to stress in adults with a background of childhood neglect (Bunea et al. 2017; Kempke et al. 2015; Lai et al. 2021). Common findings of these studies are the

importance of duration and severity of neglect and the moderating influence of psychopathology.

Compared to the data on the effect of childhood neglect on HPA axis activity, literature on the effect on the ANS is sparse. As almost no studies so far have focused on the effects of neglect specifically, data on other forms of maltreatment will be included in the following summary. In a recent review on the effect of maltreatment on the ANS response to stress in children by Young-Southward et al. (2020), an overall trend towards blunted cardiovascular responsivity and sympathetic activity was found. The authors further report mixed findings on vagal activity with one study finding decreased vagal withdrawal in response to stress and one study finding no effect for childhood maltreatment on vagal activity in children. In a large sample of N=27781 adults with a wide age range (18-65), Kuzminskaite et al. (2020) assessed markers of ANS activity (HR, RSA and pre-ejection period indicating SNS activity) at rest and during a stress test condition (interviews, cognitive task) and found no association with any form of childhood maltreatment including emotional and physical neglect. Sigrist et al. (2021) conducted a meta-analysis on the effect of early life maltreatment on resting-state HRV and found severity of maltreatment was negatively associated with resting-state vagal activity in older but not in younger samples. They interpret this finding as indicting an increase of the negative impact of early maltreatment on ANS function over time. The null-result of Kuzminskaite et al. (2020) might therefore be due to the wide age range of the sample.

Studies focusing on the effect of childhood maltreatment on the ANS response to stress have almost exclusively been done in samples of young adults (age<30) and the following evidence might thus not be generalizable to older samples. Nevertheless, results on ANS reactivity and childhood maltreatment have been mixed. Beilharz et al. (2020) report an increased HR reactivity and a delayed recovery after stress, indicating a shift towards sympathetic dominance, in participants reporting childhood maltreatment. Studies by Lovallo (2013) and Voellmin et al. (2015) have suggested an association of childhood maltreatment and a blunted autonomic stress response as implied by a reduced HR and systolic blood pressure reactivity. Concerning the effects on vagal responses to stress, atypical patterns of vagal regulation, illustrated by a dissociation of HR reactivity, RSA reactivity and baseline vagal activity, have been reported in young women with a background of childhood maltreatment (Dale et al. 2018).

Taken together, an association of childhood maltreatment and an altered ANS function with reduced cardiac vagal modulation is likely. For sympathetic activity however, a specific pattern of dysregulation cannot not yet be determined. It can thus be assumed that both divisions of the

stress response system, the HPA axis and the ANS, are affected by the formative impact of childhood neglect. A dysregulation of the stress system is commonly hypothesized to be a central mediating factor in the relationship of childhood neglect and type 2 diabetes in adulthood (Lown et al. 2019; Miller et al. 2011; Rich-Edwards et al. 2010). This hypothesis is further supported by studies illustrating possible pathogenic pathways involving the stress response system and disease-related pathology such as increased stress-related inflammatory activity (Schreier et al. 2020) metabolic disturbances (Pervanidou and Chrousos 2012) and markers of allostatic load (Widom et al. 2015) in samples with a history of neglect and maltreatment. However, the association between type 2 diabetes and childhood neglect with a dysregulated stress response has to date not been studied. The second aim of the present study is therefore to investigate the association of childhood neglect and the cardiac autonomic as well as the HPA axis' response to stress.

An additional line of evidence has been focused on the effect of childhood maltreatment and childhood neglect on the psychological stress response. The commonly observed dysregulation of the physiological stress response systems is reflected in reports of impaired regulatory abilities in people with a background of childhood neglect and maltreatment (Berzenski, 2019; Lovallo, 2013). Problems in emotion- and stress-regulation have hereby been shown to be a mediator for the effects of maltreatment (Hong et al. 2018), increasing the risk of engaging in maladaptive behaviors and regulation strategies such as physical inactivity and overeating (Dutcher et al. 2017; Felitti et al. 1998; Lee et al. 2014; Michopoulos et al. 2015) leading to higher rates of obesity (Shin and Miller 2012) and metabolic risk (van Reedt Dortland et al. 2012) in these groups. Psychological stress reactivity and impaired stress-regulation are thus a distinct but related pathway in the relationship between childhood neglect and type 2 diabetes. A third aim of this study was to examine the association of childhood neglect and the psychological response to stress.

3 Aims and Research Questions

The aims of the study are to investigate the cardiac autonomic stress response in type 2 diabetes patients compared to healthy controls and to test the association of self-reported childhood neglect with the physiological and psychological stress response in type 2 diabetes patients compared to healthy controls. The following research questions will be examined:

I. Does the cardiac autonomic stress response of type 2 diabetes patients differ from the cardiac autonomic stress response of healthy controls? Different aspects of the cardiac autonomic stress response will be operationalized by measuring HR and HRV. HRV will be assessed using high frequency (HF) HRV, to measure vagal modulatory activity and low frequency (LF) HRV, to measure over all cardiac autonomic modulatory activity.

   Additional Analyses: Does the cardiac autonomic stress response of type 2 diabetes patients suffering from diabetic complications differ from the cardiac autonomic stress response of healthy controls?

II. Is the cardiac autonomic stress response in type 2 diabetes patients associated with childhood emotional and physical neglect and do these associations differ between type 2 diabetes patients and healthy controls? Different aspects of the cardiac autonomic stress response will be operationalized by measuring HR, HF and LF HRV.

III. Is the stress response of the HPA axis in type 2 diabetes patients associated with childhood emotional and physical neglect and do these associations differ between type 2 diabetes patients and healthy controls? The stress response of the HPA axis will be assessed by measuring ACTH and plasma cortisol.

IV. Is the psychological stress response in type 2 diabetes patients associated with childhood emotional and physical neglect and do these associations differ between type 2 diabetes patients and healthy controls?

4 Method

The study was approved by the ethics committee of the Medical Faculty of the University of Heidelberg (S-019(2017)). Data collection took place from June 2018 to July 2019 and was done within a larger study, results of which can be found in (Buckert et al. 2022).

4.1 Sample and Recruitment

Type 2 diabetes patients were largely recruited through the diabetes outpatient clinic of the University Hospital Heidelberg. Only eligible type 2 diabetes patients, who were listed in a database of the German Center of Diabetes Research as interested in participating in research, were contacted. Type 2 diabetes patients as well as healthy control participants were also recruited via newspaper- and online adds and through flyers and posters at pharmacies, in doctor's offices in and around Heidelberg and at public lectures of the university clinic.

All participants had to be between 40 and 80 years old. Healthy control participants were recruited to match the sample of participants with type 2 diabetes regarding age and gender. Participants in the type 2 diabetes group needed to have a diagnosis of type 2 diabetes by a licensed physician and were excluded if they suffered from any other major illness apart from type 2 diabetes or diabetic complications such as cancer, neurological diseases, severe psychiatric disorders or severe heart- liver- or kidney diseases. Healthy control participants were required to have no past or current diagnosis of type 2 diabetes and were otherwise screened using the same criteria. Individuals with conditions and regular medication intake that are known to affect the physiological stress system such as Cushing's disease, autoimmune diseases, steroid-based or antidepressant medication were specifically screened for and excluded. Individuals who smoked more than 10 cigarettes a day, drank regularly more than three alcoholic beverages a day or engaged in other forms of drug use were also excluded.

4.2 Psychological Measures

Sociodemographic and basic clinical data including weight, height and current medication were assessed via self-report questionnaire. To assess childhood emotional and physical neglect, the German Version of the well-established *Childhood Trauma Questionnaire* (CTQ, Wingenfeld et al. 2010) was used. The CTQ retrospectively assesses experiences of abuse and neglect before the age of 18. The German version includes 28 Items constituting five subscales: Emotional Abuse (EA), Physical Abuse (PA), Sexual Abuse (SA), Emotional Neglect (EN), Physical Neglect (PN). Items are rated on a five-point Likert scale from "not at all" to "very often". Scales

can be used for continuous assessment of traumatic experiences in childhood (severity) as well as for categorical prevalence scores with cut-offs scores for "moderate to severe" abuse or neglect differing between scales (Häuser et al. 2011). Wingenfeld et al. (2010) have reported good reliability and internal consistency for all scales of the German version of the CTQ.

Participant's subjective psychological stress response was measured using visual analogue rating scales (VAS). Feelings of tension, as well as the appraisal of the stressful situation ("threatening", "stressful" and "a challenge") were rated on a continuous scale from 1 to 10. Translated versions of all items assessing the psychological stress response can be found in Appendix C.

To assess lifetime depression, a structured clinical interview based on the section A (affective disorders) of the *structural clinical interview for DSM IV* (SCID IV) was conducted. The SCID is seen as the current gold standard procedure for assessing psychopathology (Wittchen et al. 1997) .

4.3 Psychological Stress Paradigm

The Trier Social Stress Test (TSST, Kirschbaum et al. 1993) was used to induce a stress response. The TSST is a widely used procedure that has been shown to reliably provoke a psychological and physiological stress response in a variety of different samples including samples with a mean age of >60 (Allen et al. 2017). It combines a motivated performance task with the experience of uncontrollability within the context of social-evaluative threat. Participants receive instructions for a simulated job interview, which then takes place in a separate room in front of two "committee members" and a prominently placed camera. In the room, participants are informed that they will have to give a speech in front of the committee and that the committee members are trained to observe and analyze participant's nonverbal behavior. They are then given a five-minute preparation period during which the committee members closely watch them and take notes. During the speech and the entire duration of the stress test (ca. 14 minutes), the committee will keep a completely neutral facial expression and will not engage in any form of social interaction other than the TSST protocol. In the last part of the TSST, participants have to perform a surprise mental arithmetic task (serial subtraction of high numbers) in front of the committee. Participants are debriefed after the subsequent resting period of one hour.

4.4 Procedure and Blood Sampling

Participants were screened for eligibility via telephone. They were then sent the study information as well as the CTQ and the questionnaire on demographic data via mail to fill out at home. All participants were instructed to abstain from intense physical activity and alcohol consumption the night before study participation. They were further instructed not to eat or drink anything except water on the morning of the study and to get up at least 1.5 hours before their appointment, to avoid interference through the cortisol awakening response.

Participants arrived on site between 8:30 and 9:30 am. They were again informed about the study procedure and had the opportunity to ask questions. After they provided written, informed consent, a venal catheter was placed in participant's non-dominant arm and an electrocardiogram (ECG) logger was attached. Next, the SCID interview was conducted. Subsequently, participants filled in the first VAS and the first blood sample (T_0) was drawn. Participants then received instructions for the TSST and were accompanied to a separate room were the TSST took place. Immediately after the stress test, the second blood sample was drawn (T_1) and participants filled in the VAS including their appraisal of the stressful situation. During the following resting period, participants provided two more blood samples 30 (T_2) and 60 (T_3) minutes after the TSST as well as a third rating on the VAS 45 minutes after the TSST. After completion of the experimental protocol, participants went through a medical examination (s. 4.6) for the assessment of diabetes-associated complications. A graphical depiction of the study procedure can be found in Figure 1.

Figure 1: Study Procedure

Time	Phase	Psychological Measures	Physiological Measures
9:00	preperation		placement of venal catheter attachment of ECG logger
9:30	pre-stress phase TSST: instruction	SCID-Interview VAS 1	ECG sample 1 blood sample 1 ECG sample 2
10:00	TSST 1: job interview TSST 2: arithmetic task		ECG sample 3 ECG sample 4
10:15	post-stress phase	VAS 2	ECG sample 5, blood sample 2
10:30			ECG sample 6
10:45			blood sample 3
11:00		VAS 3	
11:15			blood sample 4
11:30	medical examination		urine sample NSS, NDS, SAS funduscopy

TSST: trier social stress test; SCID: structured clinical interview for DSM-IV; VAS: visual analogue scale
NSS: neuropathy symptom score; NDS: neuropathy disability score; SAS: survey of autonomic symptoms.
Note that the time structure displayed here depicts a model procedure.

4.5 ECG Sampling and HRV Analysis

An ambulatory, 5-lead ECG logger (Schiller Medilog AR12 Plus) was used to perform ECG recordings with a sampling frequency of 8000 Hz. Relevant events (such as the beginning and end of the stress test) were marked in the recording. Using these markers, six three-minute ECG-samples from the recording were extracted for each participant. The baseline sample was recorded while participants were in a relaxed, seated position ("baseline") approximately 15 minutes after arrival on site. The second sample ("anticipation") was recorded during the preparation period of the TSST, while participants were already seated in the TSST room. The third ("stress test 1") sample was recorded during participant's speech and the fourth ("stress test 2") during the arithmetic task. Sample five ("post-stress") was recorded in the three minutes directly after the TSST and the last sample ("recovery") 15 minutes after the TSST.

Raw ECG data were processed using Kubios HRV software version 3.3 (Kubios Oy, Kuopio, Finland) and according to the Task Force Guidelines (Malik et al. 1996). Kubios automatically marks R-waves to calculate RR intervals and create a heart period time series. The marked QRS complexes were inspected visually and edited where necessary. Technical and physiological artefacts (ectopic beats, arithmetic events) were identified visually as well as through a threshold-based correction algorithm using a threshold value of 0.35 sec. If samples consisted of more than 5% corrected or removed beats they were excluded from further analysis.

A parametric autoregressive modeling approach was used to estimate power spectral density (Porat and Marple, 1988). High frequency (HF) and low frequency (LF) HRV were extracted using the established frequency bands (HF: 0.15-0.4 Hz; LF: 0.04-0.15 Hz (Malik et al. 1996)). While LF HRV is used as an index of both parasympathetic as well as sympathetic cardiac modulatory influence, reflecting for example modulation of vasomotor tone, HF HRV is strongly related to RSA and thus indexes mainly (but not exclusively) vagal modulation of cardiac activity (Reyes del Paso et al. 2013; Shaffer et al. 2014; Shaffer and Ginsberg 2017).

4.6 Medical Examination

All participants underwent a medical examination in the diabetes outpatient clinic of the University Hospital Heidelberg to determine the presence of diabetes-associated microvascular complications (peripheral neuropathy, retinopathy, and nephropathy). Two questionnaires were used to assess symptoms of peripheral neuropathy: the Neuropathy Symptom Score (NSS) and the Neuropathy Disability Score (NDS; Young et al. 1993). The NSS consists of five items, four of which are scored from 0 (asymptomatic) to 2 (high symptom load) and one item with 0

(no) or 1(yes), and asks for patient's experiences of pain or discomfort in the legs. The maximum symptom score is nine. The NDS consist of eight items and is derived during the examination of patient's ankle reflex and temperature, pin-prick and vibration sensation at the toes. Items for reflexes are scored 0 (normal), 1 (present with reinforcement) to 2 (absent) and items for sensory modalities are scored as either 0 (present) or 1 (reduced/absent). The maximum symptom score is 10. Peripheral neuropathy was considered to be present if participants reached a symptom score ≥3 on the NDS or the NSS.

Symptoms of autonomic neuropathy were assessed using the German version of the survey of autonomic symptoms (Jost et al. 2012; Zilliox et al. 2011). The survey consists of 12 items in men and 11 items in women and inquires vasomotor, gastrointestinal, orthostatic, urinary and sudomotor symptoms as well as erectile dysfunction. Items assess symptom presence as well as symptom severity, rated on a scale from 1 ("the symptom bothers me not all") to 5 ("…bothers me a lot"). Sum scores are calculated for symptom presence (men: 0-12; women: 0-11) as well as symptom severity (men: 0-60; women: 0-55). Retinopathy was determined by funduscopy. Participants provided a urine sample for the diagnosis of nephropathy. Albuminuria was considered present at an albumin-creatinine-ratio (calculated as urinary albumin/(urinary creatinine/100)) above 30 mg/g.

4.7 Blood Analysis

Samples were analyzed in the accredited central laboratory of the Heidelberg University Hospital using standard operating procedures. Blood samples were centrifuged at 3,500 g for ten minutes. Plasma and serum samples were either analyzed directly or stored at -20 °C before analysis. ACTH levels were analyzed using a Siemens Immulite 2000 Immunoassay System (reagents kit: L2KAC2) with a sensitivity of 5.0 pg/l and inter- and intra-assay coefficients of variation below 7% and 5% respectively. Cortisol levels were analyzed on a Siemens ADVIA Centaur XPT Immunoassay System (reagents kit: 04344187) with a sensitivity of 5.5 nmol/l and inter- and intra-assay coefficients of variation below 7%.

4.8 Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows version 27 (IBM Corp., 2017). As described in 4.2, CTQ scores were used for continuous assessment of childhood abuse and neglect ("severity") as well as for a binary prevalence scores (high vs. low neglect or abuse) using a cut-off score for "moderate to severe" abuse or neglect. Hereafter,

whenever CTQ scores are used as continuous variable this will be denoted by the word "severity". Otherwise, "childhood neglect" can be understood as a binary variable.
χ^2- and *t*-tests were used to compare patients with type 2 diabetes and healthy control participants on demographic variables, BMI, glycated hemoglobin (Hba1c), lifetime depression, medication intake, autonomic symptoms, prevalence of physical and emotional neglect, severity of physical and emotional neglect as well as regarding participant's psychological stress response and appraisal of the stress test as scored on the VAS. Type 2 diabetes and patients and healthy control participants with and without a background of emotional and physical neglect were additionally compared regarding age, gender and SCID diagnoses of lifetime major depression again using χ^2- and *t*-tests.

In the following, analysis procedures for different aspects of the stress response in type 2 diabetes patients and healthy controls and their associations with childhood neglect and severity of childhood neglect will be described. Note that, depending on the respective quality and nature of the data as well as the specific research question, different statistical procedures were applied. If possible, continuous severity scores of childhood neglect were used to assess associations with neglect to preserve power. Nevertheless, in some cases (ANOVA, linear regression), childhood neglect was entered as binary variable to avoid multicollinearity and the consequent increase in standard errors and loss of power when including interaction terms (Aiken and West, 1991). Multilevel models were used whenever the data had a repeated measures structure. As multilevel analysis does not require sphericity and allows for incomplete data matrices (Snijders and Bosker, 2011) while preserving the maximum sample size, this analysis procedure proved most appropriate for the longitudinal data sets present in this study.

4.8.1 Effects of Childhood Neglect on the Psychological Stress Response: ANOCVA and Multiple Regression Analysis

Analyses of covariance (ANCOVAs) were applied to compare patients with type 2 diabetes and healthy controls with and without a background of childhood neglect regarding their appraisal of the stress test (stressful, threatening, challenging). Age, gender and lifetime major depression were controlled for. Analyses for physical neglect and emotional neglect were run separately. Physical and emotional neglect were entered as binary variables.
Effects of childhood emotional and physical neglect on self-reported tension caused by the stress test were calculated using multiple linear regression analyses. Values for change in self-

reported tension (from the baseline measurement to the measurement directly after the stress test) were used as outcome variable, rather than repeated measures raw values, as raw values still diverged significantly from the normal distribution after data transformation.
I specified one regression model testing the effect of physical neglect and one for emotional neglect respectively. Again, physical and emotional neglect were entered as binary predictors along with type 2 diabetes and the interaction between type 2 diabetes and neglect. To control for possible confounding variables, gender, age and lifetime major depression were added as additional predictors to the regression models.

4.8.2 Longitudinal Multilevel Analysis: Cardiac Autonomic Stress Response

To analyze the effects of type 2 diabetes on the cardiac autonomic stress response, I used longitudinal multilevel modelling via SPSS MIXED. HR, HF HRV and LF HRV data were transformed using log-transformation to approach normality assumptions; outliers ($-3 > z > 3$) that remained after transformation were excluded from the analyses. I controlled for the influence of age, gender, BMI and antihypertensive medication (beta blockers, ACE inhibitors, calcium channel blockers, angiotensin receptor blockers (MacIorowska et al. 2020) by entering all control variables into the multilevel models as additional predictors. Continuous predictor variables (BMI, age) were grad mean centered.
As proposed by Peugh (2010), I modeled individual HR and HRV samples (baseline, anticipation, stress test 1, stress test 2, post-stress and recovery) as level one units while participants were modeled as level two units. In multilevel analysis, level one and two can be understood as two regression equations predicting parameters of autonomic activity (HR, HF HRV and LF HRV). The level one equation contains only time as predictor as all other predictors (type 2 diabetes and control variables) refer to participants rather than individual samples and are consequently modeled as level two predictors within the level two equation. Graphical inspection of HR and HRV data suggested a curvilinear time trend. I therefore included time as linear as well as quadratic ($time^2$) effect to model a quadratic trend for time (Grimm et al. 2011). In multilevel modeling it is possible to include cross-level interactions in the model. Therefore, not only the differences between people with type 2 diabetes overall (level two) can be determined but also differences in change in HR and HRV over time. In this regard, multilevel analysis may be compared to repeated measures ANOVA.
For all three outcome variables (HR, HF HRV and LF HRV), I specified a random intercept fixed slope model. In respect of the longitudinal nature of the data I employed a first-order

autoregressive variance structure (Goldstein et al. 1994). The models each contained the effects of time, type 2 diabetes and the control variables as well as the cross-level interactions between time and type 2 diabetes and between time and each of the control variables. A simplified version of the resulting model (in this example for HR) can be specified in formal terms as follows:

$$\text{Level 1: } HR_{ij} = \pi_{i0} + \pi_{i1}(TIME)_{ij} + \pi_{i2}(TIME)_{ij}^2 + e_{ij} \qquad \text{where } e_{ij} \sim N(0,\sigma_e^2)$$

$$\text{Level 2: } \pi_{i0} = \beta_{00} + \beta_{10}(TYPE\ 2\ DIABETES)_i + u_{i0} \qquad \text{where } u_{i0} \sim N(0,\sigma_u^2)$$

Level one represents HR for individual *i* at individual measurement point *j* (within participants model). π_{i0} signifies the random intercept for participant *i*, π_{i1} denotes the regression coefficient for the linear effect of time, and π_{i2} for the quadratic effect of time. e_{ji} is the random residual term for participant *i* at measurement point *j*. Level two can be understood as the between participants model with β_{00} denoting the grand mean, β_{10} representing the regression coefficients for the effect of type 2 diabetes and u_{i0} signifying the random component in the intercept. The control variables (i.e. hypertensive medication, age, BMI and gender) are included in the level two equation in the same manner and are not displayed in the depiction above. Within the cross-level interactions between level one and two the effect of type 2 diabetes as a level two predictor on the effects of quadratically modeled time on HR (π_{i1}, π_{i2}) are examined.

4.8.2.1 Additional Analyses: Healthy Controls and Type 2 Diabetes Patients Suffering from Diabetic Complications

In an additional analysis of the autonomic stress response, healthy control participants were compared to the subgroup of type 2 diabetes patients, who suffered from at least one diabetic complication (retinopathy, peripheral neuropathy or nephropathy). I used the same analysis procedure as described in the previous section and specified one multilevel model for each outcome variable (HR, HF HRV and LF HRV) each containing (linear and quadratic) time to model the quadratic time trend, type 2 diabetes, the controls variables as well as the interactions between time and all other predictors.

4.8.3 Longitudinal Multilevel Analysis: Association of Severity of Childhood Neglect with the Cardiac Autonomic Stress Response

To examine associations of severity of childhood physical and emotional neglect with HR, HF HRV and LF HRV, I specified two extended models for each outcome variable, one assessing

associations with severity of physical neglect and one with severity of emotional neglect. The extended models were built by entering severity of (physical or emotional) neglect as centered, continuous predictor variables into the model described above in 4.8.2. I also entered the interaction between severity of neglect and type 2 diabetes as well as the respective cross-level interactions with time (severity of neglect and time, severity of neglect, type 2 diabetes and time). I added lifetime depression as an additional control variable as it becomes a relevant confounder in the context of stress and childhood maltreatment (Agorastos et al. 2020; Jin et al. 2018).

To assess whether adding neglect and the interactions with neglect to the model significantly increased the model fit, I compared the extended models to the respective baseline model described in 4.8.2. Log likelihood estimates were used as model fit indicators. The difference between log likelihood estimations of the extended models and the respective baseline model were compared to critical values derived from the χ^2-distribution to determine a significant increase in model fit.

4.8.4 Longitudinal Multilevel Analysis: Associations with Severity of Childhood Neglect with the HPA axis Stress Response

I used the same procedure as described above to examine associations with severity of childhood physical and emotional neglect with HPA axis parameters (ACTH and cortisol levels). Data on cortisol and ACTH plasma levels were transformed using log transformation to approach normality. Outliers ($-3>z>3$) that remained after transformation were excluded from the analyses. In the models of HPA axis activity, I controlled for the effects of age, gender and lifetime depression. Unlike in the analyses of autonomic activity, time was now entered as a factor which provides the opportunity to test individual change in ACTH or cortisol levels from a reference point (T_0= baseline measurement) to specific measurement points (T_1, T_2, T_3).

This more detailed approach was feasible for HPA axis parameters as there were only four measurement points.

Similar to the analyses of autonomic activity, I modeled individual measurement points (T_0, T_1, T_2, T_3) as level one units while participants were modeled as level two units. For both HPA axis parameters, I first specified a baseline model containing only type 2 diabetes, the control variables and their respective cross-level interactions with time. I subsequently built two extended models, one testing the associations with severity of physical neglect and one testing associations with severity of emotional neglect. The first extended model was built by adding severity

of physical neglect, as well as the interaction between severity of physical neglect and time, the interaction between type 2 diabetes and severity of physical neglect and the interaction between severity of physical neglect, type 2 diabetes and time to the baseline model. Similarly, I specified a second extended model, adding severity of emotional neglect, the interaction between severity of emotional neglect and type 2 diabetes as well as the interactions between severity of emotional neglect and time and severity of emotional neglect, type 2 diabetes and time to the baseline model. Both models thus assessed whether severity of neglect showed a significant association with HPA axis parameters overall or with the change in HPA axis parameters from baseline (T_0) to specific measurement points (T_1, T_2, T_3). Additionally, the models assessed whether these associations differed between healthy control participants and type 2 diabetes patients. Again, I compared the model fit of the extended models to the model fit of the respective baseline model.

5 Results

5.1 Sample

128 participants were recruited for the study. One participant retracted his participation and data of three participants had to be excluded from all analyses as they had terminated study participation during the stress test. The final sample (s. Table 1) thus included 124 participants (97% of originally included participants). In some cases, additional data had to be excluded from specific analyses due to problems with blood sampling or high artifact ratio in ECG samples. A detailed account of all excluded and missing data can be found in Appendix A.

Participants were on average 64.4 years old (*SD*=8.1) with a range from 42 to 80 years. Healthy controls and type 2 diabetes patients did not differ significantly in age or gender. However, healthy controls had completed significantly more years of school education (p=.027) and type 2 diabetes patients had a significantly higher BMI than healthy controls (p<.001). Clinical data on all participants can be found in Table 2.

Table 1: Demographic data with differences between type 2 diabetes patients and healthy control participants. Data are depicted as means (standard deviation) or n (percentage). Group differences were tested using t-test for continuous variables as well as χ^2- tests for categorical variables

	Type 2 Diabetes Patients (*n*=74)	Healthy Controls (*n*=50)	*p*
Gender	male: 46(62.2%), female: 28 (37.8%)	male: 30 (60.0%), female: 20 (40.0%)	.81
Age (years)	65.1(8.2)	63.4(7.8)	.26
School Education			.027
<10 years of education	23(31.1%)	5(10.0%)	
10 years of education	19(25.7%)	12(24.0%)	
>10 years of education	29(39.2%)	31(62.0%)	
Does not apply	3(4.1%)	1(2.0%)	
Marital Status			.62
Single	6(8.1%)	7(14.0%)	
Married	50(67.6%)	33(66.0%)	
Divorced	10(13.5%)	7(14.0%)	
Widowed	8(10.8%)	3(6.0%)	

Table 2: Clinical data with differences between type 2 diabetes patients and healthy control participants. Data are depicted as means (standard deviation) or *n* (percentage). Group differences were tested using *t*-tests for continuous variables as well as χ^2-tests for categorical variables.

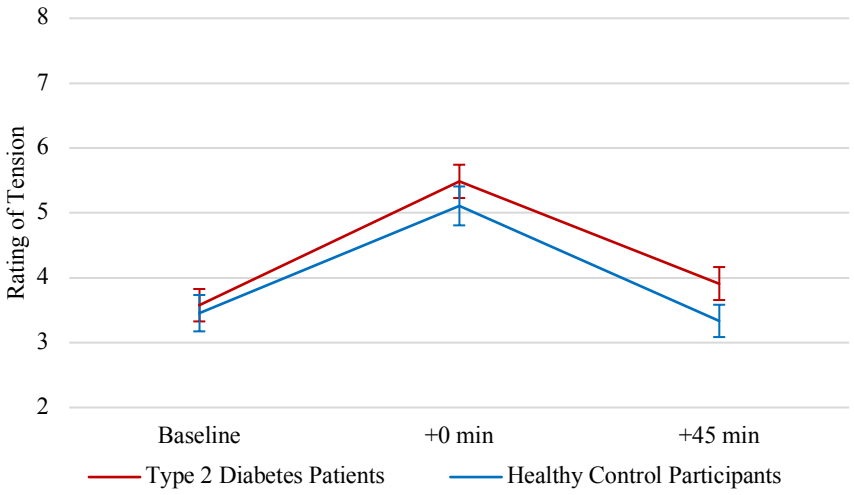
	Type 2 Diabetes Patients (*n*=74)	Healthy Controls (*n*=50)	*p*
BMI	30.2(5.7)	25.8(3.5)	<.001
Illness duration in years	13.3 (10,9)		
Hba1c	7.2(1.1)	5.5(0.4)	<.001
Medication			
Statins	29(39.2%)	8(16.0%)	.006
Insulin	21(28.4%)		
Other diabetic medication	56(75.7%)		
Beta blockers	18(24.3%)	3(6.0%)	.008
Other antihypertensive medication	44(59.5)	12(24.0%)	<.001
Diabetic Complications			
Retinopathy	12(16.2%)		
Albuminuria	20(27.0%)		
Polyneuropathy	53(71.6%)		
SAS Symptom Score (0-12)	2.3(2.4)	1.9(1.9)	.019
SAS Symptom Impact Score (0-60)	6.6(7.4)	4.2(3.7)	.26
Lifetime MD	26(35.1%)	11(22.0%)	.14

Other antihypertensive medication= ACE inhibitors, calcium channel blockers, angiotensin receptor blockers
Other diabetic medication=metformin, sulfonylureas, GLP-1 receptor agonists, gliptins, gliflozins
SAS= scale of autonomic symptoms; lifetime MD= lifetime major depression

5.2 Psychological Stress Response

Reports of tension according the VAS (s. Figure 2) increased for the whole sample from on average 3.5 (*SD*=2.1) at baseline to 5.3 (*SD*=2.2; *p*<.001) directly after the stress test. Participants appraised the TSST as *M*=6.7 (*SD*=2.2) stressful, *M*=3.0 (*SD*=2.1) threatening and *M*=6.7 (*SD*=2.5) challenging. Type 2 diabetes patients and healthy controls did not differ significantly on any of the appraisal scales, implying no differences in the subjective psychological stress response between the groups.

Figure 2: Mean ratings of tension (0-10) of type 2 diabetes patients and healthy controls before, directly after and 45 minutes after the stress test.



5.3 Cardiac Autonomic Stress Response

I analyzed differences between type 2 diabetes patients and healthy controls in autonomic reactivity using multilevel modeling. I built three models, each predicting a different indicator of autonomic activity (HR, HF HRV or LF HRV). Predictors in each model were time (quadratic trend), type 2 diabetes and the control variables (BMI, age, gender, hypertensive medication). I additionally entered interactions between time and all other predictors including the interaction of type 2 diabetes and time. The models thus tested whether the groups differed in autonomic activity overall and whether type 2 diabetes patients and healthy controls differed in change in autonomic activity over time.

5.3.1 Heart Rate

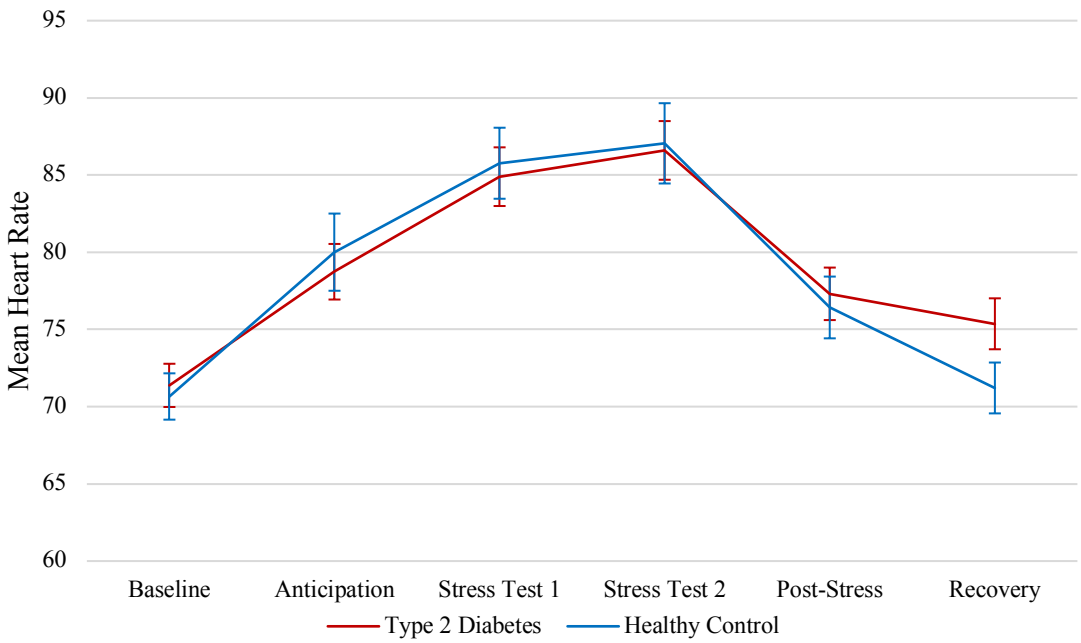
Figure 3 depicts mean HR of participants with type 2 diabetes and healthy control participants throughout the stress test. The model showed a significant, linear time trend (*est.*=0.09, $p<.001$) and a negative quadratic time trend (*est.*=-0.02, $p<.001$) reflecting the inverse U-shape of HR data over time. Type 2 diabetes had no significant main effect, implying no difference between the groups in mean HR. The model also showed no significant interaction between type 2 diabetes and linear time. For the interaction of the quadratic time trend and type 2 diabetes, the model showed a marginal, positive effect, that did not reach significance (*est.*=0.002, $p=.06$). More detailed information on predictor estimates and *p*-values can be found in Table 3.

Table 3: Multilevel model on log(heart rate) with type 2 diabetes: Estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	1.84	0.02	82.69	<.001
Time (linear)	0.09	0.01	9.49	<.001
Time (quadratic)	-0.02	0.002	-9.96	<.001
Type 2 diabetes	-0.003	0.02	-0.20	.84
Type 2 diabetes*time (linear)	-0.01	0.01	-1.48	.14
Type 2 diabetes*time (quadratic)	0.002	0.001	1.90	.060

Note: Effects of age, gender, BMI and hypertensive Medication were controlled for.

Figure 3: Mean heart rates and standard errors of type 2 diabetes patients and healthy controls before, during and after stress induction.



Note: Values depict averages of 3-minute HR-samples. Time from baseline to anticipation was on average 27 minutes. The stress test took on average 14 minutes. Time from post-stress to recovery was approximately 15 minutes.

5.3.2 Heart Rate Variability

5.3.2.1 HF HRV

Mean HF HRV of type 2 diabetes patients and healthy controls can be found in Figure 4. The model on HF HRV showed a non-significant, quadratic time trend (*est.*=0.03, *p*=.052). Type 2 diabetes had no significant main effect, implying no difference between the groups in average

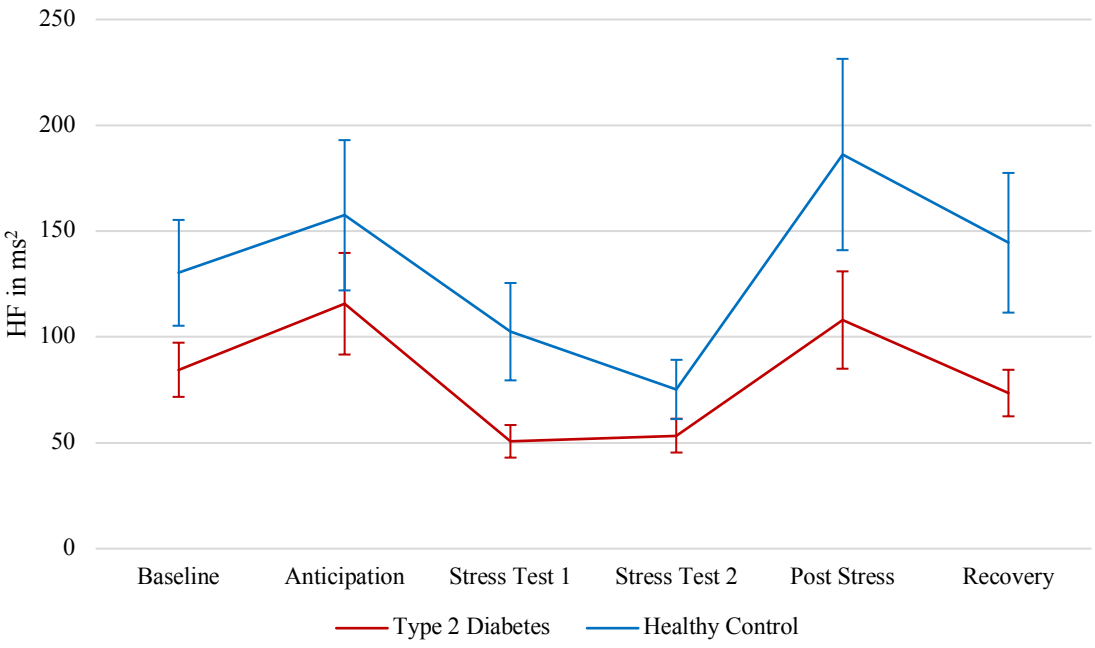
HF HRV and no interaction with linear or quadratic time, indicating no differences between the groups in change in HF HRV over time. More detailed information on predictor estimates can be found in Table 4.

Table 4: Multilevel model on log(HF HRV) with type 2 diabetes: estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	1.18	0.16	11.18	<.001
Time (linear)	-0.12	0.08	-1.48	.14
Time (quadratic)	0.03	0.02	1.95	.052
Type 2 diabetes	-0.06	0.11	-0.51	.61
Type 2 diabetes*time (linear)	-0.02	0.06	-0.30	.76
Type 2 diabetes*time (quadratic)	0.002	0.01	0.22	.83

Note: Effects of age, gender, BMI and hypertensive Medication were controlled for.

Figure 4: Mean HF HRV and standard errors of type 2 diabetes patients and healthy controls before, during and after stress induction.



Note: Values depict averages of 3-minute HRV-samples. Time from baseline to anticipation was on average 27 minutes. The stress test took on average 14 minutes. Time from post-stress to recovery was approximately 15 minutes.

5.3.2.2 LF HRV

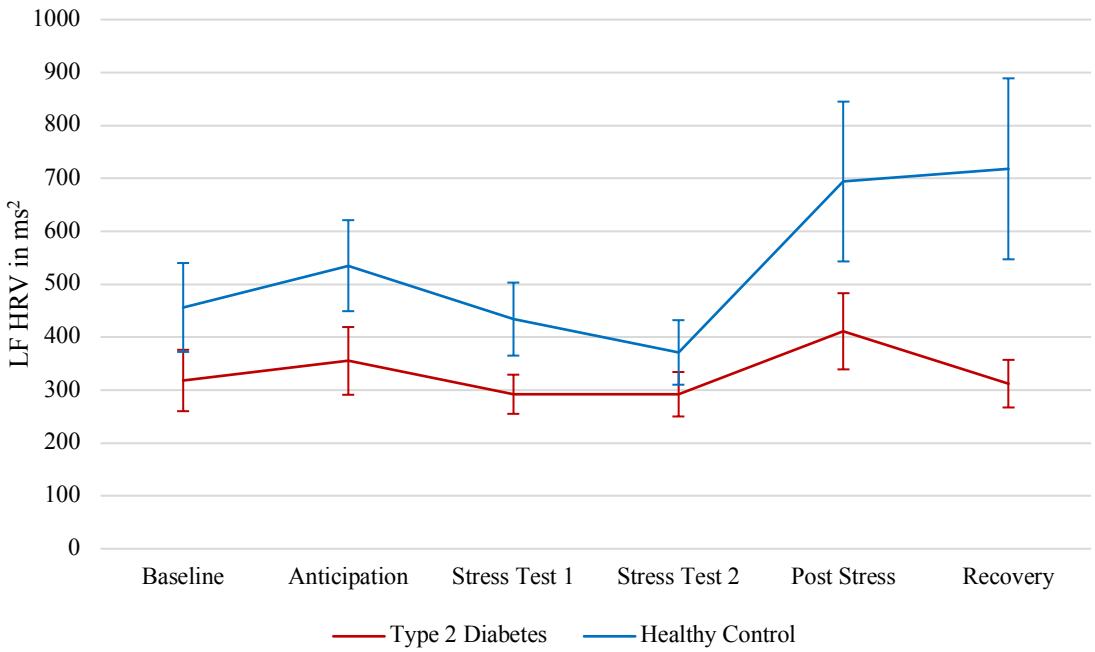
A depiction of mean LF HRV values and standard errors for participants with type 2 diabetes and healthy controls can be found in Figure 5. The model of LF HRV showed no significant time trend, indicating no significant change in LF HRV throughout the stress test. Type 2 diabetes had no significant main effect, implying no difference between the groups in mean LF HRV. For the interaction between the linear time trend and type 2 diabetes, the model showed a marginal, positive effect, that did not reach significance (*est*.=0.10, *p*=.077). The model showed a significant interaction between the quadratic time trend and type 2 diabetes (*est*.=-0.02, *p*=.044), indicating change of LF HRV over time differed between the groups with a stretched LF curve in type 2 diabetes patients. Information on predictor estimates and *p*-values can be found in Table 5.

Table 5: Multilevel model on log(LF HRV) with type 2 diabetes: estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	2.44	0.16	15.48	<.001
Time (linear)	-0.05	0.08	-0.59	.56
Time (quadratic)	0.02	0.02	1.23	.22
Type 2 diabetes	-0.12	0.11	-1.10	.27
Type 2 diabetes*time (linear)	0.10	0.06	1.77	.077
Type 2 diabetes*time (quadratic)	-0.02	0.01	-2.02	.044

Note: Effects of age, gender, BMI and hypertensive Medication were controlled for.

Figure 5: Mean LF HRV and standard errors of type 2 diabetes patients and healthy controls



Note: Values depict averages of 3-minute HRV-samples. Time from baseline to anticipation was on average 27 minutes. The stress test took on average 14 minutes. Time from post-stress to recovery was approximately 15.

5.3.3 Additional Analyses: Comparison of Healthy Controls and Type 2 Diabetes Patients Suffering from Diabetic Complications

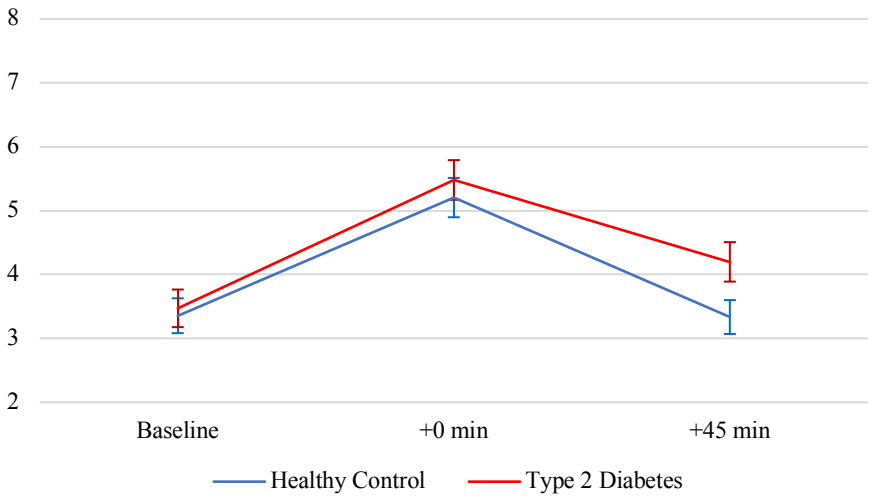
In an additional analysis of the autonomic stress response (published in Monzer et al. 2022), I compared healthy control participants to the subgroup of type 2 diabetes patients, who suffered from at least one diabetic complication (retinopathy, peripheral or neuropathy). This subgroup consisted of n=51 type 2 diabetes patients, thus excluding 23 type 2 diabetes patients. I used the same analysis procedure as described in the previous section and specified one multilevel model for each outcome variable (HR, HF HRV and LF HRV) each containing (linear and quadratic) time, type 2 diabetes, the controls variables as well as the interactions between time and all other predictors.

5.3.3.1 Psychological Stress Response

Independent sample t-tests showed no differences in self-reported tension (s. Figure 6) between participants with type 2 diabetes with complications and healthy control participants at baseline (p=.78) or directly after the stress test (p=.53). 45 minutes after the stress test however,

participants with type 2 diabetes with complications reported significantly higher levels of psychological tension (t_{94}=2.11, p=.04, d=0.43). This difference in tension at recovery was not present when the complete sample of type 2 diabetes patients was compared to healthy controls (s. 5.2).

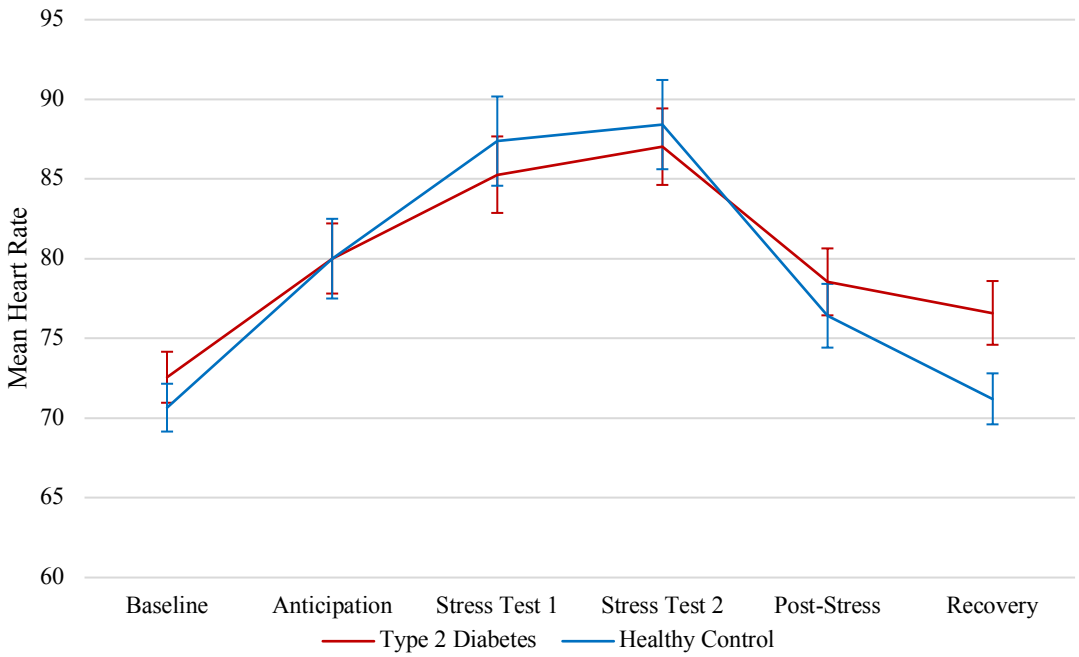
Figure 6: Mean ratings of tension (0-10) of type 2 diabetes patients with complications and healthy controls before, directly after and 45 minutes after the stress test.



5.3.3.2 Heart Rate

Figure 7 depicts mean HR and standard errors of participants with type 2 diabetes with diabetic complications and of healthy control participants. Similar to the model described in 5.3.1, this model showed a significant, linear time trend (*est*.=0.08, p<.001) and a negative quadratic time trend (*est*.=-0.02, p<.001). Again, type 2 diabetes had no significant main effect, implying no difference between the groups in mean HR. However, this model showed significant interactions between type 2 diabetes and linear (*est*.=-0.02, p=.02) as well as quadratically modeled time (*est*.=0.003, p=.02) that were not present in the model comparing healthy controls to the complete sample of type 2 diabetes patients. Thus, revealing a significant difference between the groups in HR over time, with type 2 diabetes patients with complications showing a slightly flatter, stretched HR curve.

Figure 7: Mean heart rates and standard errors of type 2 diabetes patients suffering from diabetic complications and healthy controls



Note: Values depict averages of 3-minute HR-samples. Time from baseline to anticipation was on average 27 minutes. The stress test took on average 14 minutes. Time from post-stress to recovery was approximately 15 minutes.

5.3.3.3 Heart Rate Variability

5.3.3.3.1 HF HRV

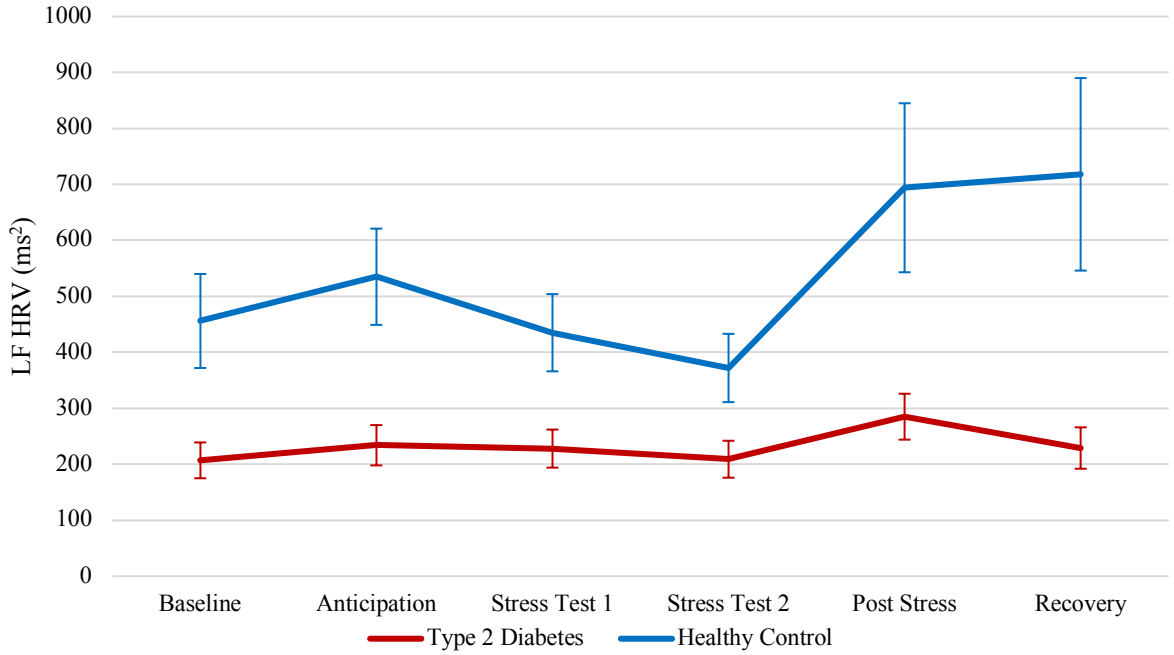
The results of this model were largely similar to the model reported in 5.3.2.1. However, the quadratic time trend was significant in this model (*est.*=0.04, *p*=.03). Additionally, type 2 diabetes now had a non-significant, negative main effect (*est.*=-0.21, *p*=.089) but, as in 5.3.2.1, no significant interaction with linear or quadratic time.

5.3.3.3.2 LF HRV

Mean LF HRV values for participants with type 2 diabetes suffering from diabetic complications and healthy controls can be found in Figure 8. Similar to the model described in 5.3.2.2., this model showed no significant time trend, indicating no significant change in LF HRV over time. However, in this model type 2 diabetes had a significant, negative main effect (*est.*=-0.27, *p*=.030) on LF HRV, implying overall lower LF HRV in type 2 diabetes patients suffering from diabetic complications compared to healthy controls, a difference that was not present when

comparing healthy controls to the complete sample of type 2 diabetes patients. As the model in 5.3.2.2., this model showed the non-significant interaction between type 2 diabetes and linear time (*est.*=0.11, *p*=.075). The interaction between type 2 diabetes and quadratic time was reduced to a non-significant trend (*est.*=-0.22, *p*=.063) in this model.

Figure 8: Mean LF HRV and standard errors of type 2 diabetes patients suffering from diabetic complications and healthy controls



Note: Values depict averages of 3-minute HR-samples. Time from baseline to anticipation was on average 27 minutes. The stress test took on average 14 minutes. Time from post-stress to recovery was approximately 15 minutes.

5.4 Physical and Emotional Childhood Neglect: Prevalence, Severity and Associations with Sample Characteristics

As described in 4.2, CTQ scores were used for continuous assessment of childhood abuse and neglect ("severity") as well as for a binary prevalence score (high vs. low neglect or abuse) using a cut-off score for "moderate to severe" abuse or neglect. In the following, whenever CTQ scores are used as continuous variable this will be denoted by the word "severity". Otherwise, "childhood neglect" can be understood as a binary variable.

Average scores in severity of physical neglect as assessed by the CTQ were 7.8 (*SD*=2.6) for type 2 diabetes patients and 7.5 (*SD*=2.2) for healthy controls. The groups did not differ in severity of physical neglect ($t(122)= -0.66$, $p=0.51$). For severity of emotional neglect, mean scores were 11.6 (*SD*=5.9) for type 2 diabetes patients and 10.1 (*SD*=4.0) for healthy controls. The difference between the groups in severity of emotional neglect did not reach significance ($t(122)=-1.71$, $p=0.90$). When applying the cut-off to compute prevalence scores, experiences of physical neglect were similarly common in both groups (patients with type 2 diabetes: 18 (24.3%); healthy control participants:12 (24.0%), $\chi^2(1, N=124)=0.00$, $p=.57$). Emotional neglect on the other hand was descriptively more common in patients with type 2 diabetes, with 18 type 2 diabetes patients (24.3%) and 7 healthy control participants (14%) reporting emotional neglect. However, the difference did not reach significance ($\chi^2(1, N=124)=1.98$, $p=.119$) likely due to the small sample size. Out of the described subgroup of those participants who report childhood neglect, 11 type 2 diabetes patients (15%) and 3 healthy controls (6%) reached the cut-off for both forms on neglect.

More details on CTQ scores and differences between the groups can be found in Appendix B.

There was no significant difference in average severity scores on the remaining CTQ scales (severity of emotional abuse, sexual abuse and physical abuse) between the groups. When applying the cut-off, sexual abuse was the most common form of abuse with 12 (16.2%) type 2 diabetes patients and 7 (14.0%) healthy control participants reporting sexual abuse. Type 2 diabetes patients and healthy controls did not differ regarding the prevalence of physical, emotional or sexual abuse.

I additionally compared type 2 diabetes patients and healthy controls with and without a background of emotional and physical neglect regarding age and gender and lifetime major depression using independent sample *t*- and χ^2-tests. In type 2 diabetes patients, participants with and

without a background of emotional or physical neglect did not differ in age, gender or diagnoses of lifetime major depression.

In healthy controls however, participants with a background of physical neglect were on average M=5.5 (SE= 2.5) years older than those without (t(48)=2.20, p=.03). Physical neglect was also slightly more common in men, with 10 men and 2 women reporting physical neglect, while emotional neglect was slightly more common in women, with 5 women and 2 men reporting emotional neglect. But, for both kinds of neglect, the gender difference did not reach significance (χ^2(1, N=50)=3.58, p=.058; χ^2(1, N=50)=3.35, p=.067). The probability for a lifetime diagnosis of major depression was increased in healthy controls with a background of physical neglect with 5 of the 11 healthy controls reporting physical neglect also having the diagnosis of lifetime major depression (χ^2(1, N=50)=4.10, p=.043).

5.5 Association of Childhood Neglect with the Acute Stress Response

5.5.1 Associations of Childhood Neglect with the Psychological Stress Response

ANCOVA was applied to compare patients with type 2 diabetes and healthy controls with and without a background of childhood neglect regarding their appraisal of the stress test. Childhood neglect was hereby used as binary variable. The analysis did not show a significant effect for type 2 diabetes, physical neglect or the (physical and emotional) neglect*type 2 diabetes interactions on any of the appraisal scales (stressful, threatening, a challenge). However, emotional neglect showed a significant, negative main effect on participants' rating of the stress test as a challenge (p=.013, η2=.06) as well as threatening (p=.030, η2=.04), implying that participants (patients with type 2 diabetes and healthy controls) with a background of emotional neglect experienced the stress test as less of a challenge and felt more threatened than those without a background of emotional neglect. Emotional neglect showed no effect on other appraisal scales.

I tested the effect of childhood physical and emotional neglect on the change in self-reported tension using two linear regression models (s. Table 6 for a depiction of all relevant predictor estimates). In the model testing the effect of physical neglect (R^2=.13), the interaction between type 2 diabetes and physical neglect significantly predicted change in self-reported tension (β=0.45, p=.006). Neither type 2 diabetes nor physical neglect alone showed a significant main effect. The second model (R^2=.10), testing the effect of emotional neglect, showed a similar result, with the interaction between emotional neglect and type 2 diabetes being the only

significant predictor (β=0.40, *p*=.031) and again neither type 2 diabetes nor emotional neglect significantly predicting change in self-reported tension.
Figures 9 and 10 illustrate results of the regression analyses, showing the psychological stress response in self-reported tension separated by moderate to severe neglect and type 2 diabetes.

Data on the prevalence and severity of childhood neglect in this sample as well as on the associations of childhood neglect with the psychological stress response have been published in Monzer et al. (2021)

Table 6: Regression on change in self-reported tension by physical neglect, R^2=.13

Parameter	*b*	*SE*(*b*)	β	*T*	*p*
Intercept	1.52	0.55		2.76	.007
Type 2 diabetes	-0.68	0.63	-0.11	-1.07	.29
PN	-0.81	1.04	-0.12	-0.77	.44
PN*type 2 diabetes	3.68	1.32	0.45	2.79	.006

Regression on change in self-reported tension by emotional neglect, R^2=.10

Intercept	1.46	0.51		2.88	.005
Type 2 diabetes	-.41	0.61	-0.07	-0.67	.50
EN	-.95	1.30	-0.13	-0.73	.47
EN*type 2 diabetes	3.3	1.50	0.40	2.19	.03

PN: Physical neglect, EN: Emotional neglect, *b*: unstandardized regression coefficient, β: standardized regression coefficient

Figure 9: Self-reported tension levels before and after stress induction in patients with type 2 diabetes and healthy controls with high and low levels of childhood physical neglect. Depicted are mean values and standard errors.

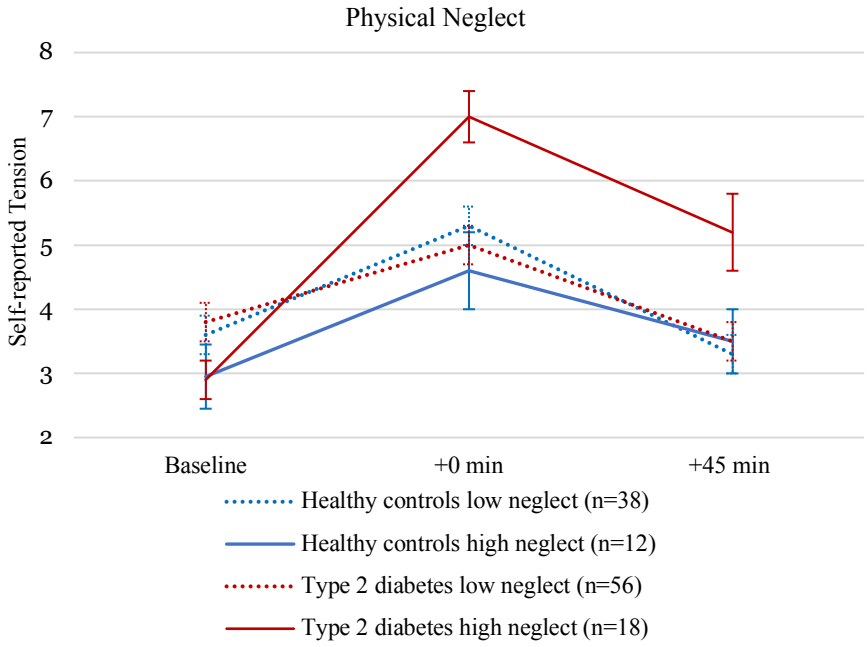
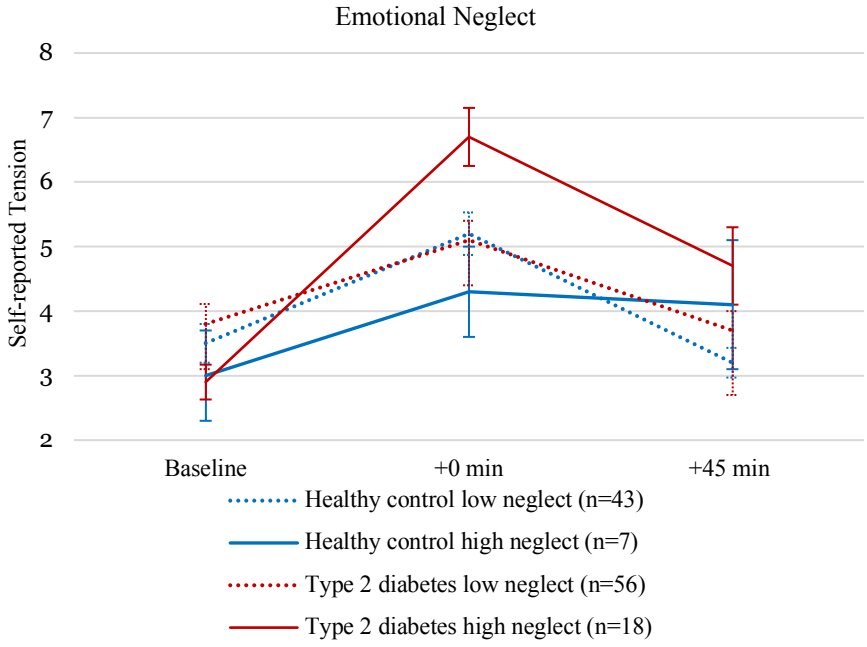


Figure 10: Self-reported tension levels before and after stress induction in patients with type 2 diabetes and healthy controls with and without the experience of "moderate to severe" childhood emotional neglect. Depicted are mean values and standard errors.



5.5.2 Associations of Severity of Childhood Neglect with the Cardiac Autonomic Stress Response

Similar to the previous analyses of autonomic activity (s. 5.3), I analyzed associations of severity of neglect and type 2 diabetes with HR and HRV using multilevel modeling. Note that, as denoted by the word "severity", childhood neglect was entered as continuous variable in these analyses.

For each parameter of autonomic activity (HR, HF HRV, LF HRV) I specified two models: one model testing associations of autonomic activity with severity of physical neglect and a second model testing associations with severity of emotional neglect. Models were built by including (quadratically modeled) time, severity of (physical or emotional) neglect, the interaction between severity of neglect and 5ype 2 diabetes and the respective interactions with time into the baseline models of autonomic activity described in 5.3.1 and 5.3.2. Models thus assessed whether severity of neglect showed a significant association with parameters of autonomic activity overall or with the change in autonomic activity over time. Additionally, models assessed whether these associations differed between healthy controls participants and type 2 diabetes patients. To assess whether including severity of neglect into the models of autonomic activity significantly increased the model fit, I compared the model fit of the baseline models to the model fit of the extended models.

In the following, results of these analyses will be summarized.

5.5.2.1 Heart Rate

Adding severity of physical or emotional neglect did not significantly increase the model fit for HR (p>.05). The extended models showed no significant effects for severity of physical neglect but when severity of emotional neglect was included in the baseline model the pattern of results changed (s. Table 7). The quadratic time trend for type 2 diabetes that was present in the baseline model (5.3.1) disappeared in this extended model. The model showed a significant association of severity of emotional neglect with HR over the linear (*est.*=-0.003, p=.002) as well as the quadratic time trend (*est.*=0.001, p<.001), indicating an association of reported severity of emotional neglect and a flatter, stretched HR curve over time across all participants. This association between severity of emotional neglect and HR differed between type 2 diabetes patients and healthy control participants: The interaction between type 2 diabetes and severity of emotional neglect was associated with HR over the linear (*est.*=0.003, p=.030) as well as the quadratic time trend (*est.*=-0.001, p=.010). That is, in type 2 diabetes patients, higher severity of

emotional neglect was associated with a more compressed HR curve compared to healthy controls.

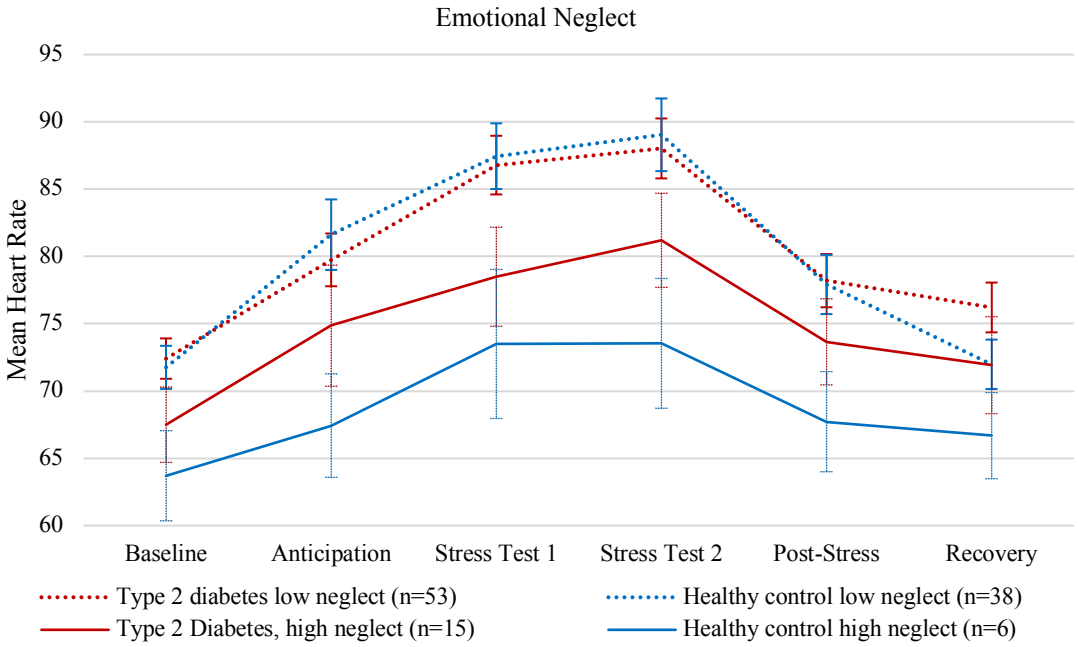
Figure 11 illustrates the relationship between severity emotional neglect, type 2 diabetes and HR over time. Please note that the grouping of the sample according to high and low reports of physical neglect was done for visualization purposes only and does not reflect the analysis procedure described here.

Table 7 Multilevel model on log(heart rate) with type 2 diabetes and severity of emotional neglect: Estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	1.83	0.02	83.50	<.001
Time (linear)	0.08	0.01	9.02	<.001
Time (quadratic)	-0.02	0.002	-9.41	<.001
Type 2 diabetes	0.001	0.02	0.07	.95
Type 2 diabetes*time (linear)	-0.01	0.01	-1.02	.31
Type 2 diabetes*time (quadratic)	0.002	0.001	1.40	.18
EN	-0.004	0.003	-1.37	.18
EN*time (linear)	-0.003	0.001	-3.16	.002
EN*time (quadratic)	0.001	0.0002	3.54	<.001
EN*type 2 diabetes	0.002	0.003	0.58	.57
EN*type 2 diabetes*time (linear)	0.003	0.001	2.18	.030
EN*type 2 diabetes*time (quadratic)	-0.001	0.0002	-2.560	.010

Note: Effects of age, gender, BMI and hypertensive Medication were controlled for. EN= severity of emotional neglect.

Figure 11: Mean heart rates and standard errors in patients with type 2 diabetes and healthy controls with high and low levels of childhood emotional neglect before, during and after stress induction



Note: Values depict averages of 3-minute HR-samples. Time from baseline to anticipation was on average 27 minutes. The stress test took on average 14 minutes. Time from post-stress to recovery was approximately 15 minutes.

5.5.2.2 Heart Rate Variability

Including severity of physical or emotional neglect into the models of HRV (HF, LF) did not significantly increase the model fit (p>.05). In the models of HF HRV, neither form of neglect alone, or in interaction with type 2 diabetes, showed a significant association. The same was true when severity of physical neglect was included in the model of LF HRV. The addition of severity of emotional neglect led to a slight change in the pattern of results as the negative interaction of type 2 diabetes and quadratic time found in the baseline model (5.3.2.2.) was now reduced to a non-significant trend (*est.*: -0.02, p=.082). Additionally, the extended model (s. Table 8) showed a negative trend, that did not reach significance (*est.*: -0.003, p=.078) for the interaction of severity of emotional neglect and the quadratic time trend.

Table 8: Multilevel model on log(LF HRV) with type 2 diabetes and severity of emotional neglect: Estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	2.44	0.16	15.33	<.001
Time (linear)	-0.03	0.08	-0.33	.74
Time (quadratic)	0.02	0.02	0.93	.35
Type 2 diabetes	-0.12	0.11	-1.11	.27
Type 2 diabetes*time (linear)	0.09	0.06	1.54	.13
Type 2 diabetes*time (quadratic)	-0.02	0.01	-1.75	.082
EN	0.004	0.02	0.18	.86
EN* time (linear)	0.02	0.01	1.57	.12
EN*time (quadratic)	-0.003	0.002	-1.77	.077
EN*type 2 diabetes	-0.01	0.02	-0.29	.78
EN*type 2 diabetes*time (linear)	-0.02	0.01	-1.42	.16
EN*type 2 diabetes*time (quadratic)	0.004	0.002	1.64	.10

Note: Effects of age, gender, BMI and hypertensive Medication were controlled for. EN= severity of emotional neglect

5.5.3 Associations of Childhood Neglect with the Stress Response of the HPA Axis

Data on the associations of childhood neglect with the stress response of the HPA axis have been published in Monzer et al. (2021).

Similar to the analyses of autonomic activity, I analyzed associations of severity of neglect and type 2 diabetes with HPA axis parameters (ACTH and cortisol) using longitudinal multilevel modeling. For each HPA axis parameter I built three models: a baseline model including all predictors except those of interest (severity of neglect and the interactions with severity of neglect), a second model testing associations with severity of physical neglect and a third model testing associations with severity of emotional neglect. To assess whether including severity of neglect into the model significantly increased the model fit, I compared the model fit of the baseline model to the model fit of the extended models. Unlike in the analyses of autonomic activity, time was now entered as a factor (T_0, T_1, T_2, T_3). This more detailed analysis was possible, as there were only four measurement points for HPA axis parameters (s. 4.8.4). Therefore, estimates for time and interactions with time in these models reflect the change and differences in change in HPA axis parameters from a reference point (T_0= baseline measurement) to specific measurement points (T_1, T_2, T_3).

5.5.3.1 ACTH

Baseline Model

The baseline model of ACTH plasma levels, which included time, type 2 diabetes and the control variables (lifetime major depression, age, gender and BMI) as predictors, showed a significant effect of time at T_1 (*est*.: 0.47; p<.001) implying an increase in ACTH levels directly after the stress test as compared to T_0. Type 2 diabetes was not significantly associated with ACTH levels overall or over time.

Physical Neglect

To test the associations of severity of physical neglect with ACTH levels, I included severity of physical neglect as well as the interaction between severity of physical neglect and type 2 diabetes and the respective interactions with time (severity of physical neglect and time; severity of physical neglect, type 2 diabetes and time) in the baseline model. This model thus assessed whether severity of physical neglect showed a significant association with ACTH levels overall or with the change in ACTH levels from baseline to specific measurement points. Additionally, this model assessed whether these associations differed between healthy control

participants and type 2 diabetes patients. Please refer to Table 9 for a depiction of all relevant predictors in this model.

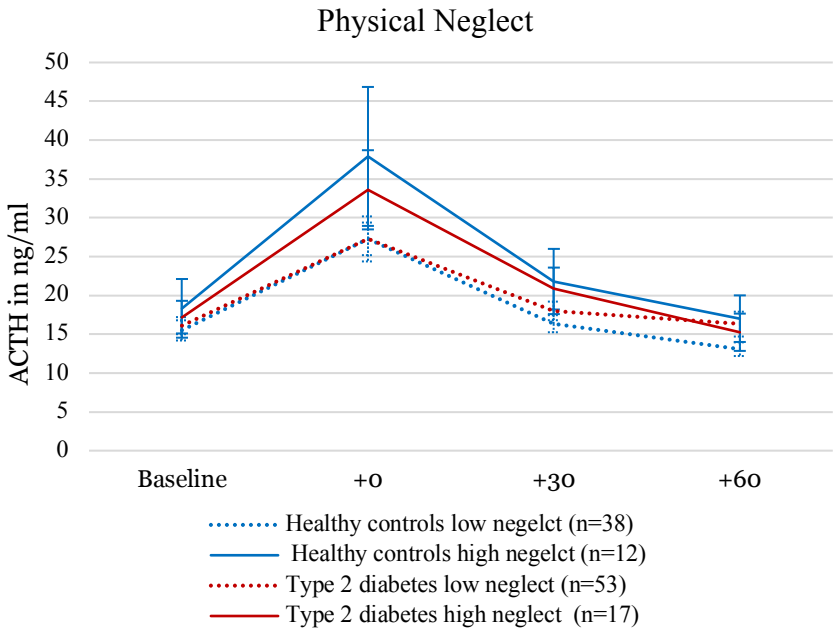
The extended model did not fit the data significantly better than the baseline model ($(-2LL_{(Baseline)}=262.5)- (-2LL_{(Physical\ Neglect)}=255.7)=6.8<\chi^2(8)=15.5$). Similar to the baseline model, this model showed a significant increase in ACTH levels directly after the stress test (*est*.=0.46, $p<.001$) as compared to T_0. There was no significant association of severity of physical neglect with ACTH levels and no significant association with ACTH levels for the interaction of type 2 diabetes and severity of physical neglect overall or over time (s. Table 9 for more details on predictor estimates). Figure 12 illustrates the relationship between severity of physical neglect, type 2 diabetes and ACTH levels. Please note that the grouping of the sample according to high and low reports of physical neglect was done for visualization purposes only and does not reflect the analysis procedure described here.

Table 9: Multilevel model on log(ACTH) with type 2 diabetes and severity of physical neglect: Estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	2.70	0.07	37.18	<.001
T1	0.46	0.06	8.19	<.001
T2	0.09	0.07	1.36	.18
T3	-0.10	0.07	-1.48	.14
Type 2 diabetes	0.02	0.09	0.23	.82
Type 2 diabetes*T1	0.01	0.07	0.19	.85
Type 2 diabetes*T2	-0.004	0.09	-0.05	.96
Type 2 diabetes*T3	0.04	0.09	0.44	.66
PN	-0.01	0.03	-0.48	.64
PN*T_1	-0.02	0.02	-0.78	.44
PN*T_2	-0.002	0.03	-0.07	.95
PN*T_3	0.01	0.03	0.39	.70
PN*type 2 diabetes	0.04	0.04	1.15	.25
PN*type 2 diabetes*T_1	0.04	0.03	1.20	.23
PN*type 2 diabetes*T_2	0.01	0.03	0.20	.85
PN*type 2 diabetes*T_3	-0.03	0.04	-0.77	.44

PN= physical neglect; T_1=+0 min, T_2=+30 min, T_3=+60 min; Effects of age, gender, BMI and lifetime major depression were controlled for.

Figure 12: ACTH levels before and after stress induction in patients with type 2 diabetes and healthy controls with high and low levels of childhood physical neglect. Depicted are mean values and standard errors.



Emotional Neglect

I included severity of emotional neglect, the interaction between severity of emotional neglect and type 2 diabetes and the respective interactions with time (severity of emotional neglect and time; severity of emotional neglect, type 2 diabetes and time) in the baseline model (s. Table 10 for a depiction of all relevant predictors in this model). Again, this extended model did not fit the data significantly better than the baseline model ($(-2LL_{(Baseline)}=262.5)$- $(-2LL_{(Emotional\ Neglect)}=250.2)=12.3<\chi^2(8)=15.51$). The model showed a similar increase in plasma ACTH levels directly after the stress test as compared to T_0 (*est.*=0.46, $p<.001$). The interaction between severity of emotional neglect and type 2 diabetes showed a significant, positive association with ACTH levels overall (*est.*=0.05, $p=.010$), indicating a positive association of ACTH levels and severity of emotional neglect in patients with type 2 diabetes overall. There was no significant interaction over time, implying no association of severity of emotional neglect and ACTH secretion in type 2 diabetes patients in response to the stress test (s. Table 10 for more details on predictor estimates).

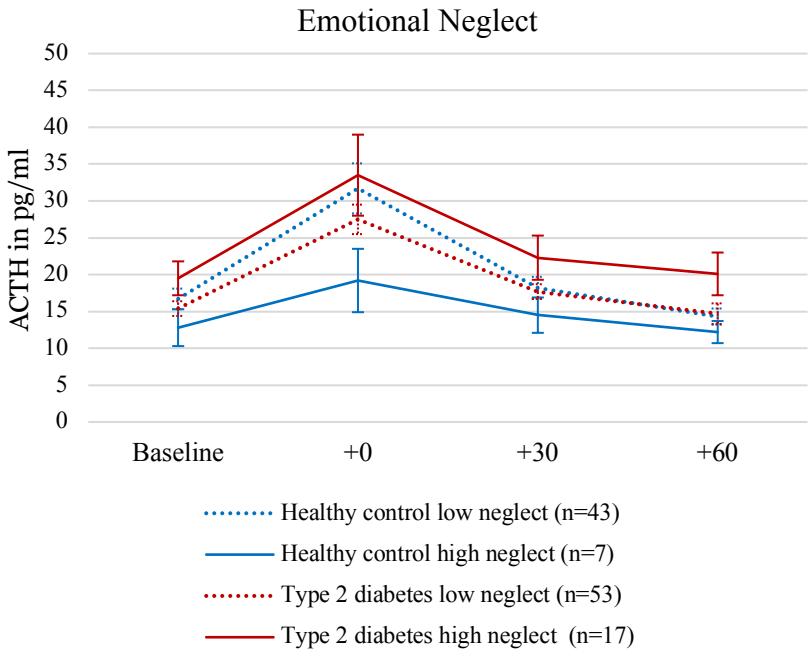
Figure 13 illustrates the relationship between severity of emotional neglect, type 2 diabetes and ACTH levels.

Table 10: Multilevel model on log(ACTH) with type 2 diabetes and severity of emotional neglect: Estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	2.66	0.07	36.86	<.001
T1	0.46	0.06	8.00	<.001
T2	0.10	0.07	1.41	.16
T3	-0.09	0.07	-1.33	.19
Type 2 diabetes	0.04	0.09	0.39	.69
Type 2 diabetes*T1	0.02	0.08	0.23	.82
Type 2 diabetes*T2	-0.01	0.09	-0.08	.94
Type 2 diabetes*T3	0.04	0.09	0.38	.71
EN	-0.03	0.02	-1.63	.10
EN*T1	-0.01	0.01	-1.13	.26
EN*T2	0.002	0.02	0.11	.91
EN*T3	0.01	0.02	0.54	.59
EN*type 2 diabetes	0.05	0.02	2.60	.010
EN*type 2 diabetes*T1	0.01	0.01	0.60	.55
EN*type 2 diabetes*T2	-0.01	0.02	-0.35	.73
EN*type 2 diabetes*T3	-0.01	0.02	-0.62	.54

EN= physical neglect; T_1=+0 min, T_2=+30 min, T_3=+60 min; Note: Effects of age, gender, BMI and lifetime major depression were controlled for.

Figure 13: ACTH levels before and after stress induction in patients with type 2 diabetes and healthy controls with high and low levels of childhood emotional neglect. Depicted are mean values and standard errors.



5.5.3.2 Cortisol

I repeated the same analysis procedure for cortisol levels.

Baseline Model

The baseline model (time with T_0 serving as reference category, type 2 diabetes and the control variables) for plasma cortisol levels showed a significant increase from T_0 to T_1 directly after the stress test (*est.*=0.25, $p<.001$) as well as from T_0 to T_2 30 min after the stress test (*est.*=0.15, $p=.01$). Type 2 diabetes had no significant main effect on cortisol levels, but a significant, positive association with a stronger increase of cortisol levels from T_0 to T_1 (*est.*=0.16, $p=.003$).

Physical Neglect

I built the extended model, by including severity of physical neglect, the interaction of severity of physical neglect and type 2 diabetes and the respective interactions with time (severity of physical neglect and time, severity of physical neglect, type 2 diabetes and time). The model thus assessed associations of severity of physical neglect with cortisol levels overall, with the change in cortisol levels from baseline (T_0) to specific measurement points (T_1, T_2, T_3) and whether these associations differed between healthy controls participants and type 2 diabetes patients. Please refer to Table 11 for a depiction of all relevant predictors.

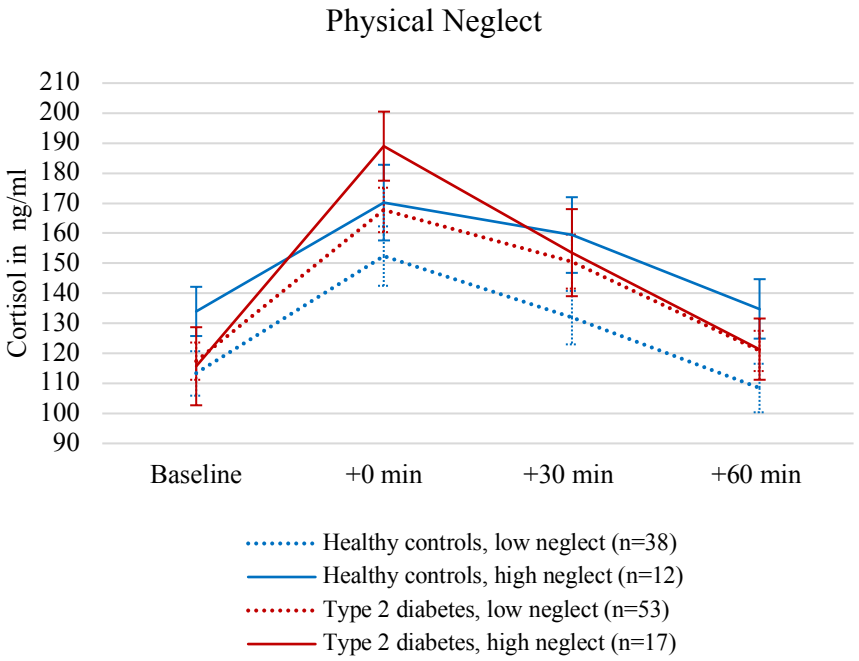
The extended model did not fit the data significantly better than the baseline model ($(-2LL_{(Baseline)}=27.1)-(-2LL_{(Physical\ Neglect)}=14.9)=12.2<\chi^2(8)=15.51$). It estimated a similar increase of cortisol levels from T_0 to T_1 directly after the stress test (*est.*=0.24, $p<.001$) and from T_0 to T_2, 30 min after the TSST (*est*.=0.14, $p=.008$) as well as a stronger increase from T_0 to T_1 for patients with type 2 diabetes (*est*.=0.16, $p=.002$). Severity of physical neglect as well as the interaction between type 2 diabetes and severity of physical neglect showed no significant association with cortisol levels. But for the interactions with time, the model revealed a significant, positive association of severity of physical neglect and change in cortisol levels from T_0 to T_1 in patients with type 2 diabetes (*est.*:0.05, $p=.013$). This result pattern indicates no association between cortisol secretion and severity of physical neglect in type 2 diabetes patients overall but a positive association between severity of physical neglect and a stronger increase in cortisol levels in response to the stress test in type 2 diabetes patients. That is a stronger cortisol response with higher severity of physical neglect in type 2 diabetes patients (s. Table 11 for more details on predictor estimates). Figure 14 illustrates the relationship between severity of physical neglect, type 2 diabetes and cortisol levels.

Table 11: Multilevel model on log(cortisol) with type 2 diabetes and severity of physical neglect: Estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	4.73	0.06	81.75	<.001
T1	0.24	0.04	6.07	<.001
T2	0.14	0.05	2.69	.008
T3	-0.05	0.06	-0.88	.38
Type 2 diabetes	-0.003	0.08	-0.05	.97
Type 2 diabetes*T1	0.16	0.05	3.16	.002
Type 2 diabetes*T2	0.05	0.07	0.71	.48
Type 2 diabetes*T3	0.04	0.08	0.49	.63
PN	0.03	0.02	1.22	.22
PN*T_1	-0.03	0.02	-1.54	.13
PN*T_2	-0.03	0.02	-1.41	.16
PN*T_3	-0.01	0.03	-0.29	.77
PN*type 2 diabetes	-0.03	0.03	-0.89	.38
PN*type 2 diabetes*T_1	0.05	0.02	2.50	.013
PN*type 2 diabetes*T_2	0.03	0.03	1.23	.22
PN*type 2 diabetes*T_3	0.01	0.03	0.40	.69

PN= physical neglect; T_1=+0 min, T_2=+30 min, T_3=+60 min; Note: Effects of age, gender, BMI and lifetime major depression were controlled for.

Figure 14: Cortisol levels before and after stress induction in patients with type 2 diabetes and healthy controls with high and low levels of childhood physical neglect. Depicted are mean values and standard errors.



Emotional Neglect

I included severity of motional neglect, the interaction between severity of emotional neglect and type 2 diabetes, and the respective interactions with time (emotional neglect and time; emotional neglect, type 2 diabetes and time) in the baseline model to build the extended model (s. Table 12 for a depiction of all relevant predictors in this model).

The extended model showed a similar pattern of results and did not fit the data significantly better than the baseline model ($(-2LL_{(Baseline)}=27.1)- (-2LL_{(Emotional\ Neglect)}=19.0)=8.1<\chi^2(8)=15.51$). The increase of cortisol plasma levels from T_0 to T_1 directly after the stress test (*est.*=0.23, $p<.001$) and from T_0 to T_2, 30 minutes after the stress test (*est.*=0.13, $p=.014$) remained significant as well as the positive association of type 2 diabetes with the stronger increase of cortisol level from T_0 to T_1 (*est*.=0.17, $p=.002$). Severity of emotional neglect showed a negative trend for the increase of cortisol levels from T_0 to T_1 that did however not reach significance (*est*.=-0.02, $p=.074$). The model also showed a non-significant, positive trend for the increase in cortisol levels from T_0 to T_1, (*est*.=0.02, $p=.056$), indicating a positive association between severity of emotional neglect and a slightly stronger increase in cortisol levels in response to the stress test in type 2 diabetes patients compared to healthy controls (s. Table 12 for more details on predictor estimates).

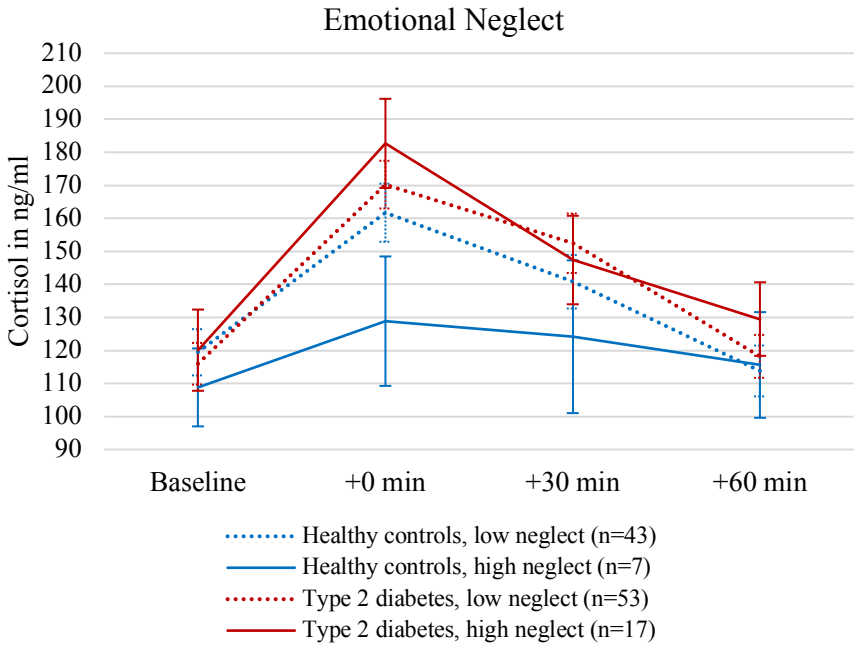
Figure 15 illustrates the relationship between severity of emotional neglect, type 2 diabetes and cortisol levels. Please note that the grouping of the sample according to high low reports of emotional neglect was done for visualization purposes only and does not reflect the analysis procedure described here

Table 12: Multilevel model on log(cortisol) with type 2 diabetes and severity of emotional neglect: estimates of fixed effects

Parameter	Estimate	*SE*	*t*	*p*
Intercept	4.73	0.06	80.09	<.001
T1	0.23	0.04	5.80	<.001
T2	0.13	0.05	2.47	.014
T3	-0.06	0.06	-0.97	.33
type 2 diabetes	-0.01	0.08	-0.12	.91
type 2 diabetes*T1	0.17	0.05	3.20	.002
type 2 diabetes*T2	0.06	0.07	0.82	.42
type 2 diabetes*T3	0.04	0.08	0.56	.57
EN	0.01	0.01	0.99	.32
EN*T1	-0.02	0.01	-1.79	.074
EN*T2	-0.02	0.01	-1.72	.09
EN*T3	-0.01	0.01	-0.83	.41
EN*type 2 diabetes	-0.01	0.02	-0.47	.64
EN*type 2 diabetes*T1	0.02	0.01	1.92	.056
EN*type 2 diabetes*T2	0.02	0.01	1.11	.27
EN*type 2 diabetes*T3	0.01	0.02	0.67	.50

EN= physical neglect; T_1=+0 min, T_2=+30 min, T_3=+60 min; Note: Effects of age, gender, BMI and lifetime major depression were controlled for.

Figure 15: Cortisol levels before and after stress induction in patients with type 2 diabetes and healthy controls with high and low levels of childhood emotional neglect. Depicted are mean values and standard errors.



6 Discussion

6.1 Main Results

The aim of this study was to investigate two, currently understudied areas concerning the stress response in type 2 diabetes: the cardiac autonomic stress response and the relationship of the psychological and physiological stress response with self-reported experiences of childhood neglect. I compared the cardiac autonomic stress response of type 2 diabetes patients and healthy controls. When the full sample of type 2 diabetes patients (those with and without diabetic complications) was compared to healthy controls, the only difference between the groups was a flatter, slightly stretched curve of LF HRV over time in type 2 diabetes patients. However, when only type 2 diabetes patients with diabetic complications were compared to healthy controls, I found a flatter, stretched HR curve indicating a blunted HR response and a reduced HR recovery that was reflected in the psychological stress response, as well as generally reduced LF HRV in type 2 diabetes patients with complications. Differences in the cardiac autonomic stress response can therefore primarily be assumed for type 2 diabetes patients with diabetic complications.

I investigated the association of self-reported childhood neglect with the psychological and physiological stress response in type 2 diabetes patients compared to healthy controls. For the psychological stress response and the stress response of the HPA axis, associations between childhood neglect and the HPA axis stress response were only present in interaction with type 2 diabetes. In type 2 diabetes patients, a stronger psychological stress response, as indicated by an increase in self-reported tension, was positively associated with moderate to severe (physical and emotional) childhood neglect. Severity of emotional neglect - but not physical neglect – was associated with increased ACTH levels in patients with type 2 diabetes. Severity of physical neglect was associated with a stronger increase in cortisol levels in response to the TSST in type 2 diabetes patients.

The relationship was less clear for the cardiac autonomic stress response. Significant associations were only found for severity of emotional neglect and the HR response. Here, a higher severity of emotional neglect was associated with a blunted HR response across both groups. This effect was attenuated in type 2 diabetes patients as indicated by a significant, negative interaction between type 2 diabetes, emotional neglect and the quadratic time trend. Taken together, a link between the stress response and childhood neglect in type 2 diabetes patients can be assumed for specific aspects of the stress response with a more robust association for the assessed psychological and endocrine, than cardiac autonomic parameters.

6.2 Cardiac Autonomic Stress Response in Type 2 Diabetes

I conducted the analysis of the cardiac autonomic stress response twice. Once comparing the full sample of type 2 diabetes patients to healthy controls and once using a reduced sample of only those type 2 diabetes patients who suffered from diabetic complications. Differences in the cardiac autonomic stress response between type 2 diabetes patients and healthy controls were predominantly present when the reduced sample of type 2 diabetes patients with diabetic complications was used. These results emphasize the importance of considering the role of diabetic complications in this context. The results of the analysis using the reduced sample will therefore be discussed first.

When the subgroup of type 2 diabetes patients with diabetic complications was compared to healthy controls, diabetes patients showed a stress response suggestive of impaired autonomic flexibility as indicated by generally reduced LF HRV and a flattend HR curve over time suggesting an anttanuated HR reactivity and a less dynamic HR recovery. The pattern suggests an imparied ability to respond to environmental demands as well as to downregulate the physiological arousal after stressor cessation. Based on these results, an impaired cardiac autonomic response to stress can be assumed for this subgroup of type 2 diabetes patients. However, due to the cross-sectional design of this study, it is not possible to determine whether the impairment of the cardiac autonomic stress response develops in conjunction with- or as a symptom of diabetic complications such as CAN or whether it is to be understod as a risk factor for the development of complications. In the aformentiond study by Steptoe et al. (2014), type 2 diabetes patients showed a HR response that was comparable to the HR response of type 2 diabetes patients with diabetic complications in the present study. Interestingly, Steptoe et al. excluded type 2 diabetes patients with signs of CAN from their study sample. It is thus possible that this changed HR response pattern is not necessarly coincident with the symptomatic manifestation of CAN and may even develop independently. Future studies may advance this line of research by investiagting this relationship within a longitudinal study design.

The oberved attenuated HR recovery in type 2 diabetes patients with complications was reflected in participants' subjective stress recovery. Type 2 diabetes patientes showed a higher level of psychological tension 45 minutes after the stress test, suggesting the observed changes in the autonomic stress response are possibly concurrent with participants' self-perception. Of course, due to the study design, a causal relationship cannot be assumed. But this finding nevertheless shows the importance of considering the interplay of physiologal and

psychological factors in type 2 diabetes research and treatment and could be applied to expand the understanding of diabetes-related stress: while suffering from type 2 diabetes is in many ways a stessor in itself, changes in the physiological stress response may additionally impair stress regulation in type 2 diabetes patients.

While type 2 diabetes patients with complications and healthy controls differed in the HR response and in LF HRV, I found no group difference in HF HRV, independently of the presence of diabetic complications. Finding no difference at all was unexpected, as HF HRV is a well-established indicator of vagally mediated HRV and decreased vagal tone is a common finding in people with type 2 diabetes (Vinik et al. 2011b) especilly in those with complications (Khandoker et al. 2017). A possible explanation may lie in the average age of the sample. Vagal tone decreases with age even in healthy populations (De Meersman and Stein 2007). The high mean age of the sample (64 years) could have lead to a levelling of HF HRV data. Additionally, Metformin, a frequently prescribed medication in type 2 diabetes patients, has been shown to increase vagal activity (Apaijai et al. 2012). As 46 % of type 2 diabetes patients (45% in the subgroup of type 2 diabetes patients with complications) reported metformine intake, this could have further diminished given group differences. Effects of metformin intake were not specifically tested or controlled for in this study.

As opposed to HF HRV, LF HRV is used as an indicator of sympathetic as well as parasympathtic activity and was significantly decreased in type 2 diabetes patients with complications. Unlike people with other chronic conditions, people with type 2 diabetes have been shown to exhibit an overall (sympathetic as well as parasympathetic) decrease in autonomic activity (Benichou et al. 2018). The results possibly reflect this finding and indicate the difference in the cardiac autonomic response to stress in type 2 diabetes patients with complications may not mainly originate from reduced vagal tone but possibly rather from a decrease in both, parasympathetic and sympathetic activity, illustrated by LF HRV rather than HF HRV.

The result pattern I observed in diabetes patients with complications can be interpreted from two different but not neccessarily opposing points of view. On the one hand, it could be understood as showing the effects of a disease-related decrease in cardiac autonomic flexibility that is rooted in type 2 diabetes pathology. In psychophysiological stress research on the other hand, this pattern is commonly interpreted as a sign of allostatic load, implying the stress system

is unable to react dynamically to stressors as it has been worn out by long periods of chronic wear an tear. Suffering from type 2 diabetes and its complications could have acted as a chronic stressor in this subgroup. However, chronic stress could have already played a role in the development of type 2 diabetes and its complications as it has been shown that deacreased autnomic flexibilty often preceeds symptomatc manifestation of type 2 diabetes (Lee et al. 2020). Again, due to the cross-scetional design of this study, assumptions on causality cannot be drawn and of course, both, diabetes-related changes and allostatic load could be present at the same time and could even have amplifed each other. Regardless of its origin, the pattern could imply a decreased ability to dynamically adapt to and recover from stressfull situations, making this subgroup possibly more vulnerable to the effects of stress, which should be considered in type 2 diabetes treatment and future research.

The results on the cardiac autonomic stress response differed considerably when the whole sample of type 2 diabetes patients was compared to healthy controls. Including the relatively small subsample of n=23 type 2 diabetes patients without diabetic complications into the analysis diminished the differences between the groups. The only difference that remained was a changed curve of LF HRV over time in type 2 diabetes patients as indicated by a negative interaction between quadratically modelled time and type 2 diabetes. The interaction maybe better understood in the context of the non-significant time (main-) effect for LF HRV, which implies no change in LF HRV throughout the stress test across both groups. The interaction could be interpreted as showing diverging LF HRV curves over time for the two groups, resulting in a non-significant quadratic time trend when tested across both groups. Importantly however, this interpretation is rather speculative and considering the remaining results on cardiac autonomic activity, a strong effect of type 2 diabetes on the cardiac autonomic stress response for the complete sample appears unlikely. Likewise, there was no difference between healthy controls and type 2 diabetes patients in self-reported tension before or after the stress test, thus reflecting the results on the cardiac autonomic stress response.

Considering the large amount of studies reporting limited autonomic flexibility, increased HR and decreased HRV in type 2 diabetes patients, the result for the complete sample of type 2 diabetes patients is surprising. But in light of the results for the reduced sample of type 2 diabetes patients with complications, it appears likely that the assumption of decreased autonomic flexibility cannot be generalized for all type 2 diabetes patients. Those who do not develop (or have not yet developed) diabetic complications may constitute a distinct subgroup in the context of acute stress showing a divergent response pattern and therefore need to be analyzed

separately. However, due to the limited sample size of the subgroup in this study, a conclusive analysis of the stress response of this subgroup was not feasible here. The exsistence of subgroupbs within type 2 diabetes patients (e.g. those who develop complications and those who do not) has been discussed in previous research (Ahlqvist et al. 2018; Bancks et al. 2021). the results of this study add to this line of evidence and show that in the context of type 2 diabetes and stress, type 2 diabetes patients should not be considered a homogenous group

As explained earlier, this study was part of a larger study on the stress response in type 2 diabetes patitients, more results of which were published in Buckert et al. (2022). In the publication by Buckert et al. differences between type 2 diabetes patients and healthy controls regarding the blood cortisol, ACTH and noradrenaline stress responses as well as more aspects of the psychological stress response were investigated. The results showed an increased cortisol response to the TSST in type 2 diabetes patients compared to healthy controls. The interplay between the ANS and the HPA axis within the stress response is not yet fully understood. However, in healthy samples, vagal activity has been shown to have an inhibiting effect on HPA axis activty (Marca et al. 2011; Weber et al. 2010) and decreased vagally mediated HRV during stress anticipation has been associated with increased cortisol secretion during stress (Pulopulos et al. 2018). In this sample, I found no difference in vagally mediated HRV (HF HRV) between the groups that corresponds with the increased cortisol response, suggesting the described relationship may not apply for type 2 diabetes patients. Possibly, a diasese-related process or the effects of allostatic load could have led to a dissociation of the two arms of the stress systems. In the results described in Buckert et al. (2022) type 2 diabetes patients also showed lower norepinephrine levels then healthy controls after the stress test. This implies decreased sympathetic reactivity in type 2 diabetes patients and corresponds with the blunted HR response and decrased LF HRV I found in type 2 diabetes patients with complications. The results published in Buckert et al. (2022) thus complement the data found in this study and taken together, these findings illustrate the complex interplay of the physiological systems involved in the stress response especially in the presence of type 2 diabetes.

6.3 Childhood Neglect and the Stress Response in Type 2 Diabetes Patients

Experiences of childhood neglect were descriptivly more common among patients with type 2 diabetes but the difference did not meet statistical significance. Considering the given evidence for an increased risk for type 2 diabetes in samples with a background of maltreatment and

particularly childhood neglect (Huang et al. 2015), it is likely that differences between the groups did not emerge due to the relativly small sample size.

I compared type 2 diabetes patients and healthy controls with and without a background of childhood neglect regarding the prevalence of lifetime major depression and found healthy controls with a background of physical neglect were more likely to have a diagnosis of lifetime depression then those without. This association was not present in type 2 diabetes patients. The effect childhood neglect likely has on the risk for lifetime depression could have been confounded by the mutual association of type 2 diabetes and major depression with type 2 diabetes being a risk factor for major depression and vice versa (Joseph and Golden 2017).

6.3.1 The Psychological Stress Response

I found a positive association of moderate to severe emotional neglect with one aspect of the psychological stress response for both groups: participants with a background of emotional neglect were more likely to feel threatened and less likely to feel the stress test was a challenge. While the latter might seem counterintuitive at first, the connotation of the German word for challenge ("Herausforderung") is rather postive. Understanding the stress test as a "challenge" could have acted as an adaptive coping strategy, allowing particitpants to access an ambitous and efficacious rather then a helpless mindset (Miller and Kirschbaum 2019). Participants reporting childhood neglect might have been less likely to have access to this coping strategy when faced with the stressor. Comparable findings have been reported in previous studies on coping in samples with a background of childhood maltreatment (Perlman et al. 2016). Translated versions of all items assessing the psychological stress response can be found in Appendix C.

Interestingly, effects of childhood neglect on self-reported tension differed between type 2 diabetes patients and healthy control participants. In type 2 diabetes patients, moderate to severe emotional and physical neglect was associated with a stronger increase in self-reported tension caused by the TSST, while this association was not present in healthy control participants. While the appraisal items (threatening, challenging) were only answered once, immediately after stress test, psychological tension was assessed three times and is therefore presumably a more accurate measure of psychological stress reactivity. Moreover, "tension" is a wider, rather unspecific term and may be better suited to describe the physical aspect of one's emotional experience. In samples with a background of childhood neglect as well as in patients with type 2 diabetes, problems in emotional clarity and even alexithymia have been described (Aust et al.

2013; Martino et al. 2020). An item using the term “tension” could therefore have been more valid to capture the experience of this particular group.

Childhood neglect thus presumably affected the appraisal of the stress test in both groups, but only in type 2 diabetes patients this led to a stronger self-reported psychological stress response. One might speculate that healthy control participants benefit from a degree of stress resilience that could also protect them from stress-associated diseases like type 2 diabetes (Crump et al. 2016).

To date, there have been no studies specifically investigating the relationship of childhood neglect with the psychological stress response in patients with type 2 diabetes. Steptoe et al. (2014) induced mental stress in healthy control participants and type 2 diabetes patients. They reported no differences in subjective stress experience between the groups. However, assuming that increased psychological stress responses are limited to the subgroup of patients with type 2 diabetes with a background of childhood neglect, this finding does not contradict the results of this study. Additionally, the level of stress induced by Steptoe et al. (2014) may not be comparable to the stress experienced during the TSST as they used a comparatively mild, cognitive stress paradigm.

An alternative explanation for the results on the psychological stress response could lie in the difference in the number of participants who reached the cut-off for moderate to severe neglect for both forms of neglect, emotional and physical. While this was the case in 11 type 2 diabetes patients, only 3 healthy controls reached the cut-off for both forms of neglect. Assuming that having experienced both forms of neglect, rather than the combination of type 2 diabetes and childhood neglect, is in fact the critical factor in the increased psychological stress response, the result would not be indicative of a diabetes-specific effect. To conclusively investigate this assumption in future research, a sample with an equal prevalence rate of the different forms of childhood neglect among the study groups would be necessary.

6.3.2 Childhood Neglect and the Physiological Stress Response

Adding physical or emotional neglect to the models predicting the physiological stress response did not lead to a significantly increase in model fit, neither in cardiac autonomic parameters nor in HPA axis parameters. Childhood neglect may in some cases be significantly associated with the physiological stress response but associations are presumably not strong enough to significantly improve the model fit. It is possible that the relatively small variance in the CTQ scales, with the majority of participants scoring relatively low, is partly responsible for this result.

Nevertheless, this is a limitation that has to be kept in mind when interpreting the results of this study. In the following, associations of childhood neglect with the cardiac autonomic stress response will be discussed first, followed by associations with the HPA axis stress response and an attempt at integrating the results on both systems.

Associations of childhood neglect and the cardiac autonomic stress response were generally sparse. I found an association of a flatter, more stretched HR curve over time with higher reported severity of emotional neglect across both groups – that is in type 2 diabetes patients and healthy controls. This result can be understood as showing an association of a decreased autonomic flexibility with higher levels of emotional neglect. As previously discussed, decreased autonomic flexibility maybe understood as a sign of allostatic load, suggesting that with increasing severity of childhood neglect, the impact of extreme or chronic stress on the cardiac autonomic stress response system grows. Results of previous studies on childhood maltreatment and cardiac autonomic reactivity in adults are mixed. Findings of this study are in line with large studies by Lovallo et al. (2012) and Voellmin et al. (2015), that found early life adversity predicted a blunted HR response to stress, but partially contradict the results by Beilharz et al. (2020) who report increased HR responses to stress with higher CTQ scores.
Interestingly, the positive interaction between type 2 diabetes and severity of emotional neglect over (quadratically modeled) time suggests a significant difference between type 2 diabetes patients and healthy controls in the association with severity of emotional neglect and HR over time. Emotional neglect was associated with a more compressed HR curve in type 2 diabetes patients than in healthy controls, indicating the blunting effect of emotional neglect on the HR stress response, was attenuated in type 2 diabetes patients. The association of HR over time with emotional neglect was thus not only present across both groups but actually decreased among type 2 diabetes patients. Disease-related changes could have had a distorting effect on the HR response pattern associated with childhood neglect in type 2 diabetes patients. Additionally, the increased stress response of the HPA axis, that was associated with childhood neglect in type 2 diabetes patients, could also help explain this result, as these systems are closely related.

I found no significant associations of childhood neglect with LF or HF HRV. This result contradicts the recent meta-analysis by Sigrist et al. (2021) who found a negative effect of childhood maltreatment on resting state vagal activity in older samples. However, under stressful

rather than resting conditions, association might be diminished. In a study by Winzeler et al. (2017) the effect of adverse childhood experiences on HR reactivity was mediated by sympathetic rather than parasympathetic activity. This would explain why I found an association with a blunted HR response but no association in HF HRV and only a non-significant trend for the association with LF HRV. No parameter that specifically assessed sympathetic activity was included in this study and although LF HRV can be understood as a measure of both parasympathetic and sympathetic influences, effects might not be strong enough to reach significance. Future studies on childhood maltreatment and the ANS may therefore profit from focusing on parameters specifically assessing SNS activity such as pre-ejection period.

There was no association of physical neglect with any of the cardiac autonomic parameters while emotional neglect showed the described association with HR. One could hypothesize that emotional neglect has a stronger impact on the ANS than physical neglect and indeed, emotional neglect has been shown to be more strongly associated with mental disorders than physical neglect (Grummitt et al. 2021). In this particular study however, an important explanation might also lie in the nature of the sample. The vast majority of the participants were German and 60 years or older (73.0%) with one third (33.3%) of the sample being older than 70. This study cohort thus represents a demographic that was largely born in the post-war period in Germany with hunger and poverty being wide-spread. Items of the physical neglect scale such as “when I was growing up I did not have enough to eat” may therefore not only capture interpersonal trauma and parental neglect but the collective experience of many families in post-war Germany. The significant correlation between physical neglect and age (r=.26; p=.004) illustrates this issue further. Despite the severe consequences these experiences may very well still have on a child’s development, this CTQ scale might be less specific in assessing experiences of childhood maltreatment in this age group (Witt et al. 2017).
While the diabetes-specific association of childhood neglect with a stronger increase in self-reported tension in response to the stress test was not reflected in the cardiac autonomic stress response, the HPA axis’ stress response partially mirrored the psychological stress response. Similar to the psychological stress response, positive associations of severity of neglect with HPA axis parameters were limited to patients with type 2 diabetes. Consequently, these results, unlike the results on the cardiac autonomic stress response, support the assumption that a dysregulated stress response system could be a link between childhood neglect and type 2 diabetes.

Regarding the stress response of the HPA axis, associations differed between types of neglect. While severity of emotional neglect was associated with higher overall ACTH levels, severity of physical neglect showed no significant association with ACTH overall or over time. Severity of physical neglect on the other hand, was associated with a stronger cortisol response to the TSST while emotional neglect was not association with cortisol. Differential effects on HPA axis functioning depending on the type of maltreatment have been reported in previous studies and recent theoretical advances in the field suggest specific physiological and psychological consequences for different types of adversity (Kuhlman et al. 2017). The results of this study add to this line of evidence and further emphasize the importance of investigating different subtypes of maltreatment and specifically emotional and physical neglect, separately.

Associations with neglect also differed between HPA axis parameters. Emotional neglect was associated with overall increased ACTH levels in type 2 diabetes patients which could indicate a chronic state of HPA axis hyperactivity. For cortisol however, I found a significant association of severity of neglect with the increase in cortisol levels in response to the TSST suggesting HPA axis hyperreactivity rather than chronic hyperactivity. A dissociation between ACTH and cortisol has been described before (Bornstein et al. 2008) and is commonly assumed to originate in adaptive changes in receptor expression or sensitivity (Lightman et al. 2021). With regard to the particular response pattern present in the results of this study, one could assume some form of counterregulatory adaption of the adrenal cortex, as it has previously been observed by Heim et al. (2000). A desensitization of the adrenal cortex to ACTH for instance could moderate the effects of chronically increased ACTH levels on cortisol secretion during baseline conditions. This mechanism might not suffice when challenged by an acute stressor, resulting in an increased cortisol response.

For this sample of older adults, a counterregulatory adaption over the course of life is a plausible assumption. The HPA axis possesses of a high degree of plasticity and is able to adapt to chronic states of overstimulation (Yiallouris et al. 2019). Additionally, the secretory patterns of the HPA axis naturally change with aging (Goncharova 2020). Over time, these changes could amplify as well as attenuate the impact of childhood maltreatment depending on environmental factors such as the amount and chronicity of further stressors as well as individual factors, such as resilience (Nederhof and Schmidt 2012). These results therefore need to be understood within the context of a complex process of lifelong adaption and the relationship between type 2 diabetes, childhood neglect and HPA axis activity potentially differs substantially when

examined in younger samples. Nonetheless, the results of this study indicate an association between an increased HPA axis activity and childhood neglect in patients with type 2 diabetes and are in line with the suggested pathway that links type 2 diabetes and childhood maltreatment via a dysregulated stress response system.

The relationship of the HPA axis and the ANS is complex. Physiologically, these systems are highly intertwined. Injections with cortisol for example decrease cardiovagal control as indicated by reductions in HRV (Adlan et al. 2018). On the other hand, cortisol secretion has also been shown to be modulated by vagal influences (Thayer and Sternberg 2006). In the context of childhood trauma, this relationship becomes arguably even more complex, as early traumatic experiences can to lead to a desynchronization of the two systems (Ali and Pruessner 2012). The following considerations therefore need to be understood as merely speculative.
I found a diabetes-specific association between childhood neglect and an increased HPA axis stress response as well as a negative association between the HR response to stress across the whole sample, that was attenuated in type 2 diabetes patients. These results might be understood conjointly as follows. Childhood emotional neglect may have a blunting effect on the HR response, which is in line with the results of studies by Lovallo et al. (2012b) and Ouellet-Morin et al. (2019) who investigated the effects of childhood maltreatment on the HR stress response. In type 2 diabetes patients the association of increased HPA activity and childhood neglect could then have attenuated this effect, resulting in the observed HR result pattern. Importantly, this explanation is only valid if one assumes an association of childhood neglect with general HPA axis overactivity, as suggested by the result found for ACTH. If, on the other hand, one assumes an association with increased HPA axis reactivity, as suggested by the result found for cortisol, an inverse relationship is more likely, as the ANS response to stress is significantly faster than the HPA axis' response. A lack of autonomic modulation, as could be inferred from the results of the comparison between type 2 diabetes patients and healthy controls, could have led to a reduced inhibition of HPA axis activity and thus to an increased HPA axis response (Pulopulos et al. 2018). Type 2 diabetes patients reporting a higher severity of childhood neglect could thus exhibit a stress response stemming from a combination of disease-related decreased autonomic modulation and trauma-related HPA axis dysregulation. What contradicts this hypothesis is the null-result for HRV as a measure of autonomic modulatory influence. To test whether the relationship suggested above might still have merit, a measurement under real resting conditions (not in the context of anticipating a stress test), to confirm assumptions of (stress-

independent) increased HPA axis activity and decreased autonomic modulatory capacity would have been necessary.

An additional way of understanding the result pattern of both systems emerges when also considering the psychological stress response. The association of childhood neglect with an increased psychological stress response in type 2 diabetes patients was only reflected in the response of the HPA axis, not in the response of the ANS. As opposed to the stress response of the ANS, which is assumed to be rather unspecific, the stress response of the HPA axis is more strongly related to the experience of negative affect and social evaluative threat (Lovallo and Buchanan, 2016). People with a background of childhood neglect have been shown to experience social evaluative situations as more aversive and threatening (Müller et al. 2019). This increased sensitivity combined with the psychological and physiological strain of suffering from type 2 diabetes could be what becomes apparent in the association of childhood neglect with an increased psychological and HPA axis stress response in type 2 diabetes patients.

As mentioned above, these considerations on the interaction of the HPA axis and the ANS in the context of type 2 diabetes and their association with childhood neglect are merely speculative. Conclusive interpretations of the entirety of these results are not possible at this point and would require further research into the specifics of the assumed associations.

6.1. Limitations and Implications for Further Research

There are several limitations that need to be taken into consideration when interpreting the results of this study. First and foremost, the cross-sectional design does not allow any inferences on causality. While longitudinal data on some of the investigated relationships exist and can inform interpretations, the results of this study are mere associations and assumptions on causality remain speculative. Secondly, only n=7 healthy controls reported moderate to severe experiences of emotional neglect. Comprehensive conclusions on the associations with emotional neglect can thus not be drawn for this group. Future studies may use a more extensive sampling procedure, aiming for a stronger representation of this group.

Furthermore, the assessment of autonomic activity in this study does not include a specific measure of sympathetic nervous system activity. Although LF HRV is commonly understood as a measure of both, parasympathetic and sympathetic activity, its validity regarding the assessment of sympathetic activity, especially in short measurements, is still debated (Reyes del Paso et al. 2013). Further research on the stress response in patients with type 2 diabetes could profit from an additional measure of sympathetic activity such as pre-ejection period.

As mentioned earlier, another limitation of the data on the stress response is the lack of a true resting state measurement. During the baseline measurement participants were filling in questionnaires, had shortly beforehand had the venal catheter placed in their arm and were already informed that they would soon have to undergo a stress test. The validity of this baseline measurement and the resulting assumptions on stress reactivity are therefore questionable.
The comparison of the autonomic stress response between type 2 diabetes patients and healthy controls and especially the additional analysis focusing on the effect of diabetic complications is also limited by the lack of a systematic diagnosis of CAN. Although measurements of HR and HRV can be used to diagnose CAN, the measurements in the present study do not meet the recommendations for diagnosis and can thus not support claims on associations with CAN. Future studies on the autonomic stress response in type 2 diabetes should therefore include the full battery of CARTs (cardiovascular automimic reflex tests) which include HR, HRV and BP responses to deep breathing, the Valsalva maneuver and postural change (Vinik et al. 2018). A systematic diagnosis of CAN could inform further research on whether alterations of the autonomic stress response correspond primarily with CAN and its severity or if they are associated with other diabetic complications as well. Results could then help answer the question of whether the changed stress response originates mainly in disease-related changes in the ANS or in the burden of living with type 2 diabetes and its complications. Ecological momentary assessment could be a useful tool to further investigate the (psychological) effects of an altered function of the autonomic nervous system on patient's day to day life. Patients could, for example, report perceived stress levels while wearing a fitness tracker recording ECG data to simultaneously assess cardiac autonomic activity in response to daily stressors.

A limitation that was already mentioned earlier needs to be discussed in this context as well: the significantly lower level of school education among type 2 diabetes patients. Childhood neglect (Mulder et al. 2018) as well as type 2 diabetes (Espelt et al. 2012) are both linked to lower levels of education. As the applied stress test involved an arithmetic challenge, type 2 diabetes patients with a more severe background of childhood neglect could have experienced an increased stress response due to their (felt or actual) limited mathematical capability, making it a potential confounder in the relationship between type 2 diabetes, childhood neglect and an altered stress response. However, when I tested the association, I found no correlation between the level of education and self-reported tension or HPA axis parameters, making a confounding influence of level of education unlikely in this study.

Another limitation concerning the associations with childhood neglect lies in the nature of this kind of childhood trauma. Neglect tends to be a chronic form of maltreatment and is not confined to a particular event. Although the timing of traumatic experiences (infancy, early childhood or adolescence) has been shown to be critically important in the context of developmental consequences of childhood maltreatment (Dunn et al. 2018), it can often not be determined conclusively. The assessment of childhood neglect in the present study did thus not include the timing of the maltreatment and is therefore limited with regards to conclusions on sensitive developmental periods. Additionally, childhood neglect tends to be highly correlated with other forms of maltreatment such as abuse and associations are likely not unique to the experience of childhood neglect. Lastly, the assessment of the psychological stress response used in this study was limited to the described VASs and may not comprehensively cover the psychological response to the TSST. Future studies on the link between childhood maltreatment and type 2 diabetes could include a more comprehensive assessment as well as possible psychological mediators such as emotion regulation abilities.

7 Conclusions

- The results on the autonomic stress response in type 2 diabetes patients highlight the complex interplay of disease-related physiological changes and psychological factors in the relationship of type 2 diabetes and stress.
- The alterations of the ANS commonly found in people with type 2 diabetes likely affect certain aspects of the autonomic stress response. Diabetic complications play a central role in this context as the association between type 2 diabetes and an altered autonomic stress response was largely limited to type 2 diabetes patients who suffer from diabetic complications.
- Future research on the mechanisms that link stress and type 2 diabetes should be expanded to include the ANS and further investigate the relationship of disease-related alterations in the ANS and patient's subjective experience of stress and its consequences.
- This is the first study to investigate the assumption that childhood maltreatment in patients with type 2 diabetes is associated with a dysregulated stress response. The results of this study suggest that this pathway is a possible mechanism that links type 2 diabetes in adulthood to the experience of neglect in childhood.
- Associations with childhood neglect in type 2 diabetes patients are hereby specific to certain aspects of the stress response and can primarily be assumed for the stress response of the HPA axis and the psychological stress response, while associations with the cardiac autonomic stress response cannot be assumed on the basis of this study.
- Associations of childhood neglect with the stress response as well as with chronic disease are observable even in older samples.
- Future research should further investigate the stress response as a possible mechanism linking type 2 diabetes and childhood maltreatment within longitudinal study designs and should specifically recruit participants with a background of childhood neglect.

In sum, this study underlines the importance of understanding type 2 diabetes as a condition that is in its causes as well as its consequences closely connected to psychosocial factors as well as patient's individual experiences and should therefore not be treated as a solely somatic condition. Moreover, this study illustrates the close link between the experience of stress and trauma with not only mental but also physical health.

8 Summary

Background: Type 2 diabetes is a growing global health concern with prevalence rates and diabetes-associated deaths still on the rise. In an effort to gain a more comprehensive understanding of the disease, diabetes research has expanded into the field of psychological factors with one focus being chronic and traumatic psychological stress. This study addresses two currently understudied areas in this field that are of special interest in type 2 diabetes patients from a psychosomatic point of view: the cardiac autonomic stress response and the relationship of the psychological and physiological stress response with self-reported experiences of childhood neglect.

Investigating the cardiac autonomic stress response could be pivotal in understanding the relationship between stress and type 2 diabetes as the physiological stress system, i.e. the hypothalamus pituitary adrenal axis (HPA axis) and the autonomic nervous system (ANS), is closely connected to the physiological systems involved in the pathology of type 2 diabetes and its complications. Studying the relationship of the stress response with childhood neglect in type 2 diabetes patients is of special interest as childhood maltreatment is associated with an increased risk for type 2 diabetes. An altered stress response could hereby be part of the mechanism that links childhood neglect to type 2 diabetes.

Method: Physical and emotional childhood neglect was assessed in *n*=74 patients with type 2 diabetes and *n*=50 healthy control participants. The trier social stress test (TSST) was used to induce a stress response. Heart rate (HR) and heart rate variability (HRV) was measured at six measurement points before, during and after the TSST. Blood ACTH and cortisol levels were measured before, directly after as well as 30 and 60 minutes after the TSST. Participants' subjective experience was assessed before, directly after as well as 45 minutes after the TSST. I compared the psychological stress response of type 2 diabetes patients and healthy controls and used multiple regression analyses to predict the change in self-reported psychological tension. Multilevel analysis (MLA) was applied to assess the association of HR, low frequency (LF) and high frequency (HF) HRV over time with type 2 diabetes. I repeated the analysis with a reduced sample containing only those type 2 diabetes patients who suffered from diabetic complications (*n*=51). MLA was also used to test associations between the cardiac autonomic stress response (HR, LF and HF), severity of childhood neglect, and type 2 diabetes as well as the HPA axis stress response (ACTH and cortisol) severity of childhood neglect, and type 2 diabetes.

Results: When the full sample of type 2 diabetes patients (those with and without diabetic complications) was compared to healthy controls, the only difference between the groups was a slightly stretched curve of LF HRV over time in type 2 diabetes patients. However, when only type 2 diabetes patients with diabetic complications were compared to healthy controls, I found a flatter, stretched HR curve indicating a blunted HR response and a reduced HR recovery that was reflected in the psychological stress response, as well as generally lower LF HRV in type 2 diabetes patients with complications.

For the psychological stress response and the stress response of the HPA axis, associations between childhood neglect and the stress response were only present in interaction with type 2 diabetes. I found a significant association between childhood neglect and a stronger psychological stress response, a positive association of severity of emotional neglect with higher ACTH levels across all measurement points, and a positive association of physical neglect and a stronger increase in plasma cortisol in response to the TSST. For the cardiac autonomic stress response, significant associations were only found for severity of emotional neglect and the HR response. Here, a higher severity of emotional neglect was associated with a blunted HR response across both groups. This effect was attenuated in type 2 diabetes patients as indicated by a significant, negative interaction between type 2 diabetes and severity of emotional neglect.

Discussion: results of this study suggest that the alterations of the ANS commonly found in people with type 2 diabetes likely affect certain aspects of the cardiac autonomic stress response. Importantly however, this relationship is largely limited to type 2 diabetes patients who suffer from diabetic complications, emphasizing the importance of diabetic complications in this context. The results on the association of childhood neglect with the stress response in patients with type 2 diabetes show that a dysregulated stress response could be part of the mechanism that links type 2 diabetes in adulthood to the experience of neglect in childhood. A link between the stress response and childhood neglect in type 2 diabetes patients can hereby primarily be assumed for the assessed psychological and endocrine parameters (ACTH and cortisol) while associations with the cardiac autonomic stress response cannot be assumed on the basis of this study.

Interpretations of the results are limited by the cross-sectional design of this study, allowing no inferences on causality, as well as by the absence of a systematic diagnosis of cardiac autonomic neuropathy. Implications for future studies include an expansion of the research on type 2 diabetes and stress to include the ANS as well as longitudinal study designs to further investigate the stress response as a mechanism linking type 2 diabetes and childhood maltreatment.

9 Zusammenfassung

Hintergrund: Type 2 Diabetes ist eine der häufigsten chronischen Erkrankungen weltweit und die Prävalenzzahlen sowie diabetes-assoziierten Todesfälle steigen aktuell weiter an. In der Diabetesforschung wird nun auch untersucht, welche Rolle psychologische Faktoren spielen, um so ein umfassenderes Verständnis der Erkrankung zu gewinnen. Chronischer und traumatischer Stress stellen dabei Forschungsschwerpunkte dar. Die vorliegenden Studie untersucht zwei, bisher wenig erforschte, Themenbereiche, die aus psychosomatischer Sicht von besonderem Interesse bei Typ 2 Diabetes Patient*innen sind: Die Stressreaktion des autonomen Nervensystems (ANS) und der Zusammenhang zwischen der psychologischen und physiologischen Stressreaktion und Vernachlässigungserfahrungen in der Kindheit.

Weil das physiologische Stresssystem (die Hypothalamus-Hypophysen-Nebennierenrinden-Achse (HHNA) und das ANS) eng mit den physiologischen Systemen verbunden sind, die bei Typ 2 Diabetes von Bedeutung sind, könnte eine Untersuchung der Stressreaktion des ANS dazu beitragen, die Beziehung zwischen Typ 2 und Stress besser zu verstehen. Die Untersuchung einer möglichen Beziehung zwischen der Stressreaktion und Vernachlässigungserfahrungen in der Kindheit, ist von besonderem Interesse, weil bereits gezeigt werden konnte, dass Opfer von Kindesmisshandlung ein erhöhtes Risiko für Typ 2 Diabetes haben. Eine veränderte Stressreaktion könnte ein Teil des Mechanismus sein, der diesen Zusammenhang erklärt.

Methode: In *n*=74 Typ 2 Diabetes Patienten und *n*=50 gesunden Kontrollpersonen wurden körperliche und emotionale Vernachlässigungserfahrungen erfasst. Der Trier sozialer Stress Test (TSST) wurde eingesetzt um eine Stressreaktion auszulösen. Dabei wurden die Herz Rate (HR) und die Herzratenvariabilität (HRV) zu sechs Messzeitpunkten vor, während und nach dem TSST gemessen. Weiterhin wurden die ACTH- und Cortisol Blutwerte vor, nach, sowie 30 und 60 Minuten nach dem TSST gemessen. Die psychologische Stressreaktion der Teilnehmer*innen wurde vor, direkt nach, sowie 45 Minuten nach dem TSST erfragt. Die psychologische Stressreaktion der Typ 2 Diabetes Patient*innen wurde mit der der gesunden Kontrollpersonen verglichen und multiple Regressionen wurden verwendet, um die Veränderung in subjektiver Anspannung vorherzusagen. Anhand von Multilevelanalysen (MLA), wurde die Assoziation zwischen HR, Low Frequency (LF) und High Frequency (HF) HRV mit Typ 2 Diabetes über den zeitlichen Verlauf getestet. Diese Analyse wurde mit einer reduzierten Stichprobe, die nur Typ 2 Diabetes Patient*innen enthielt die an Folgeerkrankungen litten (*n*=51) wiederholt. Ebenfalls anhand von MLA wurde die Assoziation zwischen der autonomen Stressreaktion (HR, LF und HF), der Schwere früher Vernachlässigungserfahrungen und Typ 2 Diabetes sowie

der Stressreaktion der HHNA (ACTH und Cortisol), der Schwere früher Vernachlässigungserfahrungen und Typ 2 Diabetes getestet.

Ergebnisse: Der Vergleich der vollständigen Typ 2 Diabetes Gruppe (mit und ohne Folgeerkrankungen) mit gesunden Kontrollpersonen zeigte als einzigen Unterschied eine gestreckte LF HRV Kurve bei Typ 2 Diabetes Patient*innen. Als der Vergleich mit der reduzierten Stichprobe (mit Folgeerkrankungen) wiederholt wurde, zeigte sich bei Typ 2 Diabetes Patient*innen eine insgesamt niedrigere LF HRV sowie eine gestreckte HR Kurve, die auf eine schwächere Reaktivität sowie eine verlangsamte Erholung hinweist. Dieses Muster fand sich auch in der psychologischen Stressreaktion der Typ 2 Diabetes Gruppe wieder.

Assoziationen der psychologischen und der endokrinologischen (HHNA) Stressreaktion mit frühen Vernachlässigungserfahrungen fanden sich nur in Interaktion mit Typ 2 Diabetes. Dabei ergab sich ein Zusammenhang zwischen Vernachlässigungserfahrungen und einer stärkeren psychologischen Stressreaktion, eine positive Assoziation zwischen der Schwere emotionaler Vernachlässigung und insgesamt höheren ACTH Blutwerten sowie eine positive Assoziation zwischen einem stärkeren Cortisol Anstieg nach dem TSST und der Schwere körperlicher Vernachlässigungserfahrungen. Bei der autonomen Stressreaktion ergab sich nur eine Assoziation zwischen der Schwere emotionaler Vernachlässigungserfahrungen und einer gestreckten HR Kurve in beiden Gruppen. Eine negative Interaktion zwischen Type 2 Diabetes und der Schwere emotionaler Vernachlässigungserfahrungen zeigte, dass dieser Zusammenhang bei Typ 2 Diabetes Patient*innen allerdings signifikant schwächer war.

Diskussion: Auf der Basis der Ergebnisse dieser Studie kann angenommen werden, dass Veränderungen des ANS bei Typ 2 Diabetes Patient*innen bestimmte Aspekte der Stressreaktion beeinflussen. Allerdings besteht dieser Zusammenhang vor allem bei Typ 2 Diabetes Patient*innen mit Folgeerkrankungen, was die Bedeutung von Folgeerkrankungen in diesem Kontext unterstreicht. Die Ergebnisse zeigen außerdem, dass eine dysregulierte Stressreaktion bei Typ 2 Diabetes Patient*innen ein Teil des Mechanismus sein könnte, der Typ 2 Diabetes und frühe Vernachlässigungserfahrungen verbindet. Dabei kann vor allem für die gemessenen endokrinologischen und psychologischen Parameter ein Zusammenhang angenommen werden, während ein Zusammenhang mit der autonomen Stressreaktion unwahrscheinlicher ist. Wichtige Limitation dieser Studie sind das querschnittliche Studiendesign, das keine Rückschlüsse auf kausale Zusammenhänge zulässt, sowie das Fehlen einer systematischen Diagnostik autonomer Neuropathie. Weitergehende Forschung in diesem Gebiet sollte das ANS miteinschließen und longitudinale Studiendesigns verwenden.

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11 Publications, Funding, Personal Contribution to Data Acquisition and Assessment

This dissertation was part of the research project WI 4115/5-1 “Die Bedeutung einer bei Diabetes veränderten psychischen Stressreaktion auf die Freisetzung reaktiver Metabolite als mögliche Ursache diabetischer Folgeschäden“ (REMDIS), which was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) and conducted in association with SFB 1118 (project number: 236360313). I personally received a 2-year scholarship by the “Stiftung der Deutschen Wirtschaft” as well as a 2-year ideational scholarship by the “Studienstiftung des deutschen Volkes”.

I was involved in designing the study and contributed the aspect of childhood maltreatment as one focus point of the study. Recruitment and screening were organized predominantly by me with help from a student research assistant and partly by a medical doctoral candidate (CS). I collected the majority of participant data acting as the main examiner conducting the study protocol for roughly 70% of participants. All data analyses described in this dissertation project were done by me with assistance from BW, MH and KW.

The majority of the results described in this dissertation project have been published in the following papers:

1. Monzer, N., Hartmann, M., Buckert, M., Wolff, K., Nawroth, P., Kopf, S., Kender, Z., Friederich, H.C. and Wild, B. (2021). A**ssociations of Childhood Neglect With the ACTH and Plasma Cortisol Stress Response in Patients With Type 2 Diabetes.** Front. Psychiatry *12*, 986. doi:10.3389/fpsyt.2021.679693

2. Monzer, N. L., Hartmann, M., Buckert, M., Wolff, K., Nawroth, P., Kopf, S., Kender, Z., Friederich, H.C. and Wild, B. (2022). **The cardiac autonomic response to acute psychological stress in type 2 diabetes.** PLoS One *17*, e0265234. doi:10.1371/journal.pone.0265234

Publication 1 is based on the results described in 5.4, 5.5.1 and 5.5.3. Publication 2 is based on the results described in 5.3.3. All other results described in this dissertation have not been published.

The research questions of this dissertation concerning the cardiac autonomic stress response in type 2 diabetes patients and the associations between childhood neglect and the stress response in type 2 diabetes patients were conceptualized, investigated and published by me with assistance from BW and MH.

The main results of the REMEDIS study are published here:

- Buckert, M., Hartmann, M., Monzer, N., Wolff, K., Nawroth, P., Fleming, T., Streibel, C., Henningsen, N. and Wild, B. (2022). **Pronounced cortisol response to acute psychosocial stress in type 2 diabetes patients with and without complications.** Horm. Behav. *141*, 105120. doi:10.1016/j.yhbeh.2022.105120

Results of this study are described and discussed in the Discussion section of this dissertation. The publication by Buckert et al. (2022) investigates the stress response in the same sample of type 2 diabetes patients and healthy controls using the following biological parameters: ACTH, cortisol, norepinephrine, and methylglyoxal and did not include HR or HRV. This dissertation only focuses on ACTH and cortisol in association with childhood neglect (which is not included in the publication by Buckert et al. (2022)). In the publication by Buckert et al. (2022) the psychological stress response was measured using the German Multidimensional Mood Questionnaire (Mehrdimensionaler Befindlichkeitsfragebogen; MDBF) which is not included in this dissertation. There are therefore no overlaps between the results described in this dissertation and the results published in Buckert et al. (2022).

Further unrelated personal publications:

- Monzer, N., Herzog, W., Löwe, B., Zipfel, S., Henningsen, P., Rose, M. and Lehmann, M. (2019). **Reviving the Clinician Scientist: A Best Practice Model**. Psychotherapy and psychosomatics, *88*, 114-116. doi:10.1159/000495693

- Hartmann, M., Monzer, N., Schultz, J.H., Ditzen, B., Wensing, M., Schmalenberger, K. and Herzog, W. (2021). **Promoting research competence in psychosocial medicine-A new curriculum for medical students**. Z Psychosom Med Psychother *67*, 78–87. doi:10.13109/zptm.2021.67.1.78

Appendix A: Excluded and Missing Data

Table A and B show a compilation of missing or excluded data for each measurement of the key variables. Data sets of four participants with type 2 diabetes were excluded from analysis completely as they had terminated the study participation prematurely. Data of these participants are not included in Table A and B.

General reasons for the exclusion of data were intolerable deviations from the study protocol such as sizable changes in the timing of the study procedures or problems during the stress test. Additionally, I excluded statistical outliers with *z*-values -3>z>3. Data from blood samples were excluded due to problems during sampling (inefficient blood flow) or due to problems with the venal catheter. ECG data were excluded when ECG samples showed a high (>5%) artefact ratio. CTQ and VAS data were excluded due to missings in the respective questionnaires.

Table A: Number and percentage of excluded and missing data in **type 2 diabetes patients** (*n*=74)

	ACTH				Cortisol				ECG Data						EN	PN	VAS		
Measure-ment	1	2	3	4	1	2	3	4	1	2	3	4	5	6			1	2	3
Number Missings	2 (3%)	9 (12%)	11 (15%)	12 (16%)	2 (3%)	8 (11%)	10 (14%)	12 (16%)	7 (10%)	11 (15%)	8 (11%)	12 (16%)	14 (19%)	13 (18%)	1 (1%)	5 (7%)	2 (3%)	3 (4%)	3 (4%)

EN: emotional neglect, PN: physical neglect; VAS: visual analogue scale

Table B: Number and percentage of excluded and missing data in **healthy controls** (*n*=50)

	ACTH				Cortisol				ECG Data						EN	PN	VAS		
Measure-ment	1	2	3	4	1	2	3	4	1	2	3	4	5	6			1	2	3
Number Missings	2 (4%)	7 (14%)	8 (16%)	8 (16%)	2 (4%)	6 (12%)	8 (16%)	9 (18%)	6 (12%)	6 (12%)	8 (16%)	11 (22%)	9 (18%)	9 (18%)	2 (4%)	0 (0%)	1 (2%)	2 (4%)	2 (4%)

EN: emotional neglect, PN: physical neglect; VAS: visual analogue scale

Appendix B: CTQ Scores

Table C: CTQ severity scores depicted as mean values with standard deviations and prevalence of moderate to severe abuse or neglect depicted as number with percentages.

CTQ Severity Scores (5-25)	Type 2 Diabetes Patients (*n*=74)	Healthy Controls (*n*=50)	*p*
Emotional neglect	11.6(5.9)	10.1(4.0)	.090
Physical neglect	7.8(2.6)	7.5(2.2)	.51
Emotional abuse	7.2(3.1)	7.3(3.0)	.83
Physical abuse	6.4(3.2)	6.1(2.2)	.64
Sexual abuse	6.5(4.0%)	6.2(3.1%)	.64
Prevalence of Moderate to Severe Abuse or Neglect			
Emotional neglect	18(24.3%)	7(14.0%)	.12
Physical neglect	18(24.3%)	12(24.0)	.57
Emotional abuse	4(5.4%)	2(4.0%)	.54
Physical abuse	9(12.4%)	3(6.0)	.21
Sexual abuse	12(16.2%)	7(14.0%)	.49

Note: *P*-values are derived using *t*-test for continuous severity scores and χ^2-test for categorical prevalence scores. Cut-off scores for "moderate to severe" abuse or neglect vary for each scale and are based on Häuser et al. (2011).

Appendix C: VAS Items

(Translated from German)

I. Psychological Tension

The following item was answered before, directly after as well as 45 minutes after the stress test.

Please indicate how you're feeling currently! You can place your mark on or in between numbers.

How tense/relaxed are you feeling in this Moment?

1.....2.....3.....4.....5.....6.....7.....8.....9.....10
completely relaxed *very tense*

II. Appraisal of the TSST

The following items were answered directly after the stress test.

Please rate the following statements on the past situation (stress test). You can place your mark on or in between numbers.

The past situation was stressful for me.
1.....2.....3.....4.....5.....6.....7.....8.....9.....10
Not at all *very much*

I experienced the past situation as a threat
1.....2.....3.....4.....5.....6.....7.....8.....9.....10
Not at all *very much*

I experienced the past situation as a challenge
1.....2.....3.....4.....5.....6.....7.....8.....9.....10
Not at all *very much*

12 Curriculum Vitae (German)

Angaben zur Person	
Name:	Nelly Lou Monzer
Geburtsdatum:	20.02.1990
Geburtsort:	Stuttgart
Familienstand:	verheiratet
Kinder:	Keine
Adresse:	Kleinfeldstrasse 27, 68165 Mannheim

Aus- und Weiterbildung	
2021	Wieslocher Institut für systemische Lösungen: Fortbildung Psychodynamisch Imaginative Traumatherapie (PITT)
Seit 07/2018	Promotionstätigkeit an der Medizinischen Fakultät des Universitätsklinikum Heidelberg „Acute Stress and Traumatic Stress in Type 2 Diabetes Mellitus" Promotionsförderung durch die Stiftung der deutschen Wirtschaft sowie die Studienstiftung des deutschen Volkes
10/2017-07/2019	Teilnahme am Qualifizierungsprogramm „Klinische Forschung" des Deutschen Kollegium für Psychosomatische Medizin (DKPM)
Seit 01/2017	Heidelberger Institut für Psychotherapie: Ausbildung zur Psychologischen Psychotherapeutin
10/2017-10/2018	Wieslocher Institut für systemische Lösungen: Aufbaukurs: Systemische Therapie und Beratung
10/2015-07/2016	Grundkurs: Systemische Therapie und Beratung
10/2014-12/2016	Universität Heidelberg, Studiengang: M.Sc. Psychologie „Clinical and Developemental Psychology" Abschlussnote: 1,0 Masterarbeit, AG Persönlichkeitsstörungen (Universitätsklinikum Heidelberg): „Cortisol Awakening Response in Borderline- and PTSD Patients "
08/2012-12/2012	Universität Reykajvik: Auslandsstudium

11/2010	Aufnahme als Stipendiatin der Studienstiftung des deutschen Volkes
09/2010-01/2014	Universität Mannheim, Studiengang: B.Sc. Psychologie Abschlussnote: 1,5
2000-2009	Besuch des Immanuel-Kant Gymnasiums in 70771 Leinfelden, Abschlussnote: 1,5

Berufserfahrung

Seit 02/2022	Psychotherapeutin für traumatisierte Geflüchtete im Psychosozialen Zentrum (PSZ) Nordbaden, Behandlungsinitiative Opferschutz (BIOS)
08/2021-01/2022	Psychotherapeutin in Ausbildung (PPiA) auf Station Tellenbach (Gerontopsychiatrie) der Klinik für Allgemeine Psychiatrie und Psychotherapie
Seit 01/2019	Ambulanztherapeutin am Heidelberger Institut für Psychotherapie
05/2017-05/2018	Psychotherapeutin in Ausbildung (PPiA) auf Station AKM der Klinik für Allgemeine Innere Medizin und Psychosomatik
Seit 01/2017-02/2022	Wissenschaftliche Mitarbeiterin in der Klinik für Allgemeine Innere Medizin und Psychosomatik, Universitätsklinikum Heidelberg

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Lastly, I want to thank my husband. Thank you for everything, always.

14 Eidesstattliche Versicherung (Affidavit)

1. Bei der eingereichten Dissertation zu dem Thema „Acute Stress and Traumatic Stress in Type 2 Diabetes Mellitus“ handelt es sich um meine eigenständig erbrachte Leistung.

2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

3. Die Arbeit oder Teile davon habe ich bislang nicht an einer Hochschule des In- oder Auslands als Bestandteil einer Prüfungs- oder Qualifikationsleistung vorgelegt.

4. Die Richtigkeit der vorstehenden Erklärungen bestätige ich.

5. Die Bedeutung der eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt. Ich versichere an Eides statt, dass ich nach bestem Wissen die reine Wahrheit erklärt und nichts verschwiegen habe.

Place, Date doctoral candidate’s signature