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**Cognitive function
and Hypothalamic-Pituitary-Adrenal axis
in Type II Diabetes**

**Función cognitiva y eje hipotálamo-hipofiso-adrenal en la
diabetes tipo II**

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Stress and cognitive function in type II
diabetes

Estrés y función cognitiva en la diabetes tipo II

Sé firme en tus actitudes
y perseverante en tu ideal.

Solo contempla la meta
y no veas que tan difícil es alcanzarla.
Si tengo la creencia de que puedo hacerlo,
de seguro podré adquirir las capacidades para hacerlo
incluso si ahora mismo no puedo.

Mahatma Gandhi

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Dissertation Outline

Type 2 Diabetes (T2D) is considered a heterogeneous metabolic disorder that is characterised by insulin resistance and relative insulin deficiency (Umegaki, 2014). There is evidence about the relationship between stress and T2D, being stress a relevant trigger for the onset of T2D (Hackett and Steptoe, 2017; Ingrosso et al., 2022), and showing a causal association in T2D. In addition, this disease could be considered as a chronic stressor, leading to the hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis (Kokkinopoulou et al., 2021). Furthermore, it is well known that the brain structures which control the HPA axis functioning, as the hippocampus, prefrontal cortex, and amygdala, are also involved in cognitive processes, such as declarative and working memory and executive functions. Thus, it is possible that changes in the levels of cortisol, the end-product of the HPA axis, could affect the cognitive function. In addition, the chronic activation of HPA axis also produces an increase in blood glucose levels, contributing to T2D development (Boaz et al., 2013; Lundqvist et al., 2019). Therefore, it is important to increase knowledge about how stress can affect cognitive performance in older people with T2D, given the influence this association could have on the daily activities of these patients. To do so, this thesis aims to characterize the psychobiological response to acute stress, the basal HPA axis functioning and its impact on cognitive function in older people with T2D.

The first chapter of this dissertation provides a general introduction describing the characteristics of T2D, its prevalence, and main features. Additionally, in this chapter the neurological mechanisms in T2D and their relation to cognitive impairment are highlighted. It also reviews the cognitive domains most affected in people with the disease, as well as the different stages of cognitive dysfunction reported in the literature. The last part of this chapter exposes the features observed in brain function, connectivity, and structure in T2D patients.

The second chapter explains the objectives and hypotheses for the three studies that compose this doctoral thesis.

The third and fourth chapters describe two studies investigating the acute stress effects on memory performance in older men and women with T2D. More specifically, the first study addresses the psychobiological response to an acute stressor of T2D older men and women and its effects on working memory performance. In the second study, the effects of acute stress on long-term declarative memory retrieval in older participants with T2D compared to healthy participants are presented.

The fifth chapter introduces the third study that explains the diurnal cortisol cycle and its relationship with different cognitive domains (declarative and working memory, and executive functions) in T2D and healthy older participants.

Chapter six includes a general discussion of the principal findings, the strengths and limitations and future directions. The chapter seven includes the main conclusions of the present doctoral thesis, and finally, the chapter eight ends the thesis with a general summary of the thesis in Spanish.

Abbreviations

A β = Amyloid-Beta

ADA= American Diabetes Association

ANS = Autonomic Nervous System

AUCg = Area Under the Curve with respect to the Ground

AUCi = Area Under the Curve with respect to the Increase

BMI = Body Mass Index

BP = Blood Pressure

BG = Blood Glucose

cAMP = Cyclic adenosine monophosphate

CAR = Cortisol Awakening Response

CGM = Cerebral grey matter

CNS = Central Nervous System

CRH = Corticotropin-Releasing Hormone

CWM = Cerebral white matter

DCS = Diurnal Cortisol Slope

DE = Diabetic Encephalopathy

DS = Digit Span

DST = Digit Symbol Test

GRs = Glucocorticoid Receptors

HbA1c = Glycated Haemoglobin

HPA axis = Hypothalamic-Pituitary-Adrenal axis

IDF = International Diabetes Federation

LNS = Letter-Number Sequencing

MCI = Mild Cognitive Impairment

fMRI = Functional Magnetic Resonance Imaging

NA = Negative Affect

NICE = National Institute for Health and Clinical Excellence

PA = Positive Affect

PANAS = Positive and Negative Affect Scale

PNS = Peripheral Nervous System

ROS = Reactive Oxygen Species
sAA = Salivary Alpha-Amylase
SES = Socioeconomic Status
SNS = Sympathetic Nervous System
T2D = Type 2 Diabetes
TMT = Trail-Making Test
TSST = Trier Social Stress Test
VAS = Valence and Arousal Scale
WHO = World Health Organization
WM = Working Memory

Chapter 1

General Introduction

Type II diabetes: definition, prevalence and impact on cognition

Type II diabetes (T2D) is considered the most prevalent metabolic chronic disorder associated with insulin resistance and hyperinsulinemia (Chatterjee et al., 2017; Khalid et al., 2022; Umegaki, 2014). This disease is due to damage of pancreatic β cell, deficiency of insulin secretion or non-use of insulin, resulting in higher levels of blood glucose (Padhi et al., 2020).

Diabetes affects more than 500 million people worldwide, increasing to 1-31 million by 2050, according to the Global Burden of Disease (GBD, 2021). The International Diabetes Federation (IDF, 2022) has highlighted that T2D is one of the fastest growing global health emergencies of the 21st century. More than 90% of all diabetes cases worldwide are due to T2D (Ali et al., 2022).

The causes of T2D are not completely understood but has been recognized a strong link with lifestyle factors which contribute to the T2D development, in particular those related to sedentary lifestyles, physical inactivity, overweight or obesity (Dedoussis et al., 2007; Kahn et al., 2014). In addition, family history of T2D implies an increased risk of developing the disease. Along these lines, some reviews have underlined the importance of genetic factors in the development of T2D (Laakso, 2019; Lin et al., 2017). More than 400 genetic variants have been found in T2D people, with a small or moderate effect on the risk of suffer T2D (Mahajan et al., 2018). In addition to all the aforementioned causes, it must be added the increasing ageing of populations worldwide (IDF, 2022). This, together with reduced physical activity and exposure to cardiometabolic factors, has led to an increase in T2D in the older population (Khan et al., 2020). In this line, the T2D becomes a common disease in people aged 50 and older (Bommer et al., 2017), with the highest prevalence of T2D observed in those aged 75–79 (IDF, 2022). Additionally, the increased prevalence of multimorbidity is as high as 40% in older adults with T2D (Piette, 2006; Wolff et al., 2002). There is also evidence of age-associated conditions that diminish quality of life in T2D adults such as chronic pain, incontinence and falls (Laiteerapong et al., 2011).

Over time, the chronic metabolic imbalance in T2D is associated with multiple complications such as retinopathy, ketoacidosis, nephropathy, myocardial infarction, stroke, and lower limb amputation (Harding et al., 2019; IDF, 2015). The treatment of these complications causes a growing economic burden for health systems (Zhang et al., 2010) because T2D patients require greater use of medication and are more likely to require long-term care (Unwin et al., 2010).

A very important point is the relationship of T2D with mental health, specifically with depression (Farooqi et al., 2022; Lindekilde et al., 2022; Van Sloten et al., 2020). The increased risk of depression may be due to diabetes-related complications (Chireh et al., 2019), because of the psychological burden following the diagnosis of T2D, the management of hyperglycaemia or the treatment of the disease itself (Anderson et al., 2001; Engum, 2007). Considering the high prevalence of T2D and depression, regular screening of it is of great importance in T2D patients, being this recommended by clinical guidelines for diabetes management (IDF, 2005; NICE, 2009).

Also, recent literature has showed an increased risk of anxiety disorders in patients with T2D (Benmaamar et al., 2022; Mersha et al., 2022). Thus, it was reported 56% greater prevalence of anxious symptoms in those patients who were aware of the diagnosis of T2D (Arteaga-Zarate et al., 2022; Meurs et al., 2016). In these patients, elevated anxiety has been shown to be associated with worsening blood glucose levels, increased body mass index (BMI) (Balhara and Sagar, 2011) and reduced quality of life (Penckofer et al., 2012). For all the above, it is recommended to screen diabetic patients for anxiety at the time of the diagnosis as well as at follow-up appointments.

In addition to all these complications, the literature has increasingly recognized the relationship between T2D and increased risk of cognitive dysfunction (Casagrande et al., 2021; Dao et al., 2022; Umegaki et al., 2013). In this line, a longitudinal population study showed an association between elderly T2D and accelerated cognitive impairment (Hassing et al., 2004).

The risk of accelerated cognitive impairment has been observed to be 1.5-2.0 times higher in T2D patients than in healthy participants, (Biessels and Despa, 2018; Xiong et al., 2020).

Associated cognitive difficulties may have negative effects on care and disease management (Tomlin and Sinclair, 2016), leading to complications such as hypoglycaemia (Lee et al., 2017). However, T2D often presents with other comorbidities also related to cognitive impairment, making it difficult to establish a causal relationship between these processes (Li et al., 2019).

Several mechanisms could contribute to explain the relationship between T2D and cognitive impairment. Thus, the combination of vascular and neurodegenerative damage may be the cause of cognitive dysfunction in patients with T2D (Cholerton et al., 2016), although the impact of T2D on cognition have not been clearly determined (Sandhir and Gupta, 2015).

Brain vulnerability to the adverse effects of T2D has been highlighted (Carvalho et al., 2020). The brain is rich in insulin receptors in areas related to cognitive memory such as the hippocampus (Pomytkin et al., 2018), a brain area that plays a role in learning and memory (Lee et al., 2012). It has been shown that reduced brain insulin sensitivity can lead to energy failure, leading to impaired brain plasticity, nerve growth and cognitive function (Cai et al., 2017; Ng et al., 2016).

Other hypothetical mechanism underlying T2D cognitive impairment has been proposed through the hyperglycaemia mediated insulin sensitivity in the brain, which would affect synaptic plasticity in the hippocampus. This could compromise memory, potentially leading to cognitive dysfunction (Kerti et al., 2013; Spinelli et al., 2019). In this line, excessive glucose concentration in T2D has toxic effects on brain neurons. The maintenance of elevated glucose levels would lead to the formation of advanced glycation end products (AGEs), leading to high toxicity effects on neurons (Yamagishi et al., 2007).

Microvascular abnormalities also affect the brain in T2D patients (Van Sloten et al., 2020), due to hypertension that could cause arterial stiffness (Climie et al., 2019). This could explain why the brain is most often affected by

microvascular diseases in T2D (Stehouwer, 2018), contributing to an increased risk of cognitive dysfunction in patients with T2D (Feinkohl et al., 2019; Reijmer et al., 2013; Van den Berg et al., 2009).

To perform the self-management tasks of T2D, it is important to know that cognitive skills remain preserved (Tomlin and Sinclair, 2016). However, the literature has demonstrated impairment in multiple cognitive domains in T2D patients (Abbatecola et al., 2010; Dyer et al., 2020; Strachan et al., 2009), presented through impaired attentional (Cukierman-Yaffe et al., 2009; Messier et al., 2010), declarative memory (DM) (Geijselaers et al., 2015; Li et al., 2021; Ravona-Springer et al., 2014), working memory (WM) (Backeström et al., 2021; Dyer et al., 2020; Monette et al., 2014; Palta et al., 2014) and Executive Function (EF) (Biessels and Despa, 2018; Casagrande et al., 2021; Cui et al., 2014; Moheet et al., 2015; Munshi et al., 2006; Sadanand et al., 2016), especially in those who are older and have T2D for longer (Velayudhan et al., 2010).

Attention refers to the observation and alertness state that allows us to become aware of what is happening in our environment (Ballesteros, 2002). Attentional difficulties have been demonstrated in patients with T2D (Manschot et al., 2006; Moheet et al., 2015). In this line, Ruis et al. (2009) found that older T2D performed significantly worse on attention. In addition, in a meta-analysis (Pelimanni and Jehkonen, 2018) and several reviews (Xue et al., 2019; Wang et al., 2021), worse performance in T2D patients was also indicated. Adequate attention allows ignoring information or detecting life-threatening changes in the environment. Therefore, attention is considered a cognitive domain of great importance (Katsuki and Constantinidis, 2014).

Concerning DM, alterations in verbal DM have been reported in patients with T2D (Yau et al., 2014). DM is an important learning system (Ullman and Pullman, 2015) that refers to the acquisition and retrieval of episodes, facts and events that allow for contrasting and comparing evoked material (Squire, 2004). Literature has reported lower performance on immediate and delayed verbal recall in T2D patients (Debling et al., 2006; Grodstein et al., 2001; Yau et al., 2014). However, in a more recent longitudinal study, worse performance on the

short and long-term memory were not statistically significant after adjustment for vascular risk factors (Xie et al., 2022).

WM is a cognitive process for the manipulation and temporary storage of information in the brain, being important for performing many complex tasks (Baddeley et al., 1996, 2003). WM was disrupted in T2D patients without complications, when compared with healthy controls, in cross-sectional (Backeström et al., 2021; Cansino et al., 2021; Mythri et al., 2017; Zhang et al., 2016) and longitudinal studies (Xie et al., 2022), as well as in a comprehensive review (Kim, 2019).

EF are the set of control processes responsible for planning, reasoning, coordinating, problem solving sequencing and cognitive operations monitoring (Hull et al., 2008; Qiu et al., 2006). Several studies have evidenced impairments on a variety of executive tasks in older adults with T2D. Thus, Van Harten et al. (2007) found worse results in EF, assessed by TMT-B (cognitive flexibility) and the Stroop subtest (part III) (inhibitory control). In addition, Yeung et al. (2009) assessed inhibitory control using the Stroop subtest (part III). The Color Trails 2 was included for assessing the same domain, but without the influence of language. Participants with T2D performed worse than healthy controls on the Color Trails 2, although no differences were observed in Stroop tests. Furthermore, in another study.

In addition, longitudinal studies showed an unfavorable evolution of cognitive performance over the years, in older patients with T2D, compared to healthy people (Fontbonne et al., 2001). Furthermore, T2D presented greater decline in the instrumental activities of daily living compared to non-T2D people (Kuo et al., 2005). In sum, dysexecutive syndrome may occur in older T2D individuals, enhancing the risk for impacting on the self-care of these patients.

With all this in mind, it is important to take into account which are the potential neurological mechanisms in T2D that could lead to the aforementioned cognitive dysfunction.

Neurological mechanisms in T2D: relationship with cognitive impairment

As mentioned in the previous section, cognitive dysfunction is accepted as a common and important complication of T2D (Biessels and Despa, 2018; McCrimmon et al., 2012). T2D is associated with different stages of cognitive dysfunction. In this line, in prediabetic stages, cognitive decline may result in expressed cognitive complaints by the patient, without affecting activities of daily living (Koekkoek et al., 2015). Regarding the more severe stages of cognitive dysfunction, it has been suggested that patients with T2D have a higher risk of Alzheimer's disease (AD) and dementia compared to healthy people (Biessels and Despa, 2018; Exalto et al., 2013; Gudala et al., 2013). Some biological mechanisms through which diabetes may increase the risk of dementia and AD have been postulated: vascular mechanisms, toxic effects of hyperglycaemia, brain insulin resistance or low brain glucose metabolism (Michailidis et al., 2022), but none have been clearly demonstrated (Cole et al., 2007; Peila et al., 2002).

In this type of patients, abnormal brain structure and function has been found (Kang et al., 2022; Li et al., 2021). The studies conducted to evaluate cerebral grey matter (CGM) and white matter (CWM) reported significantly reduced CGM volume in cortical regions, including the middle occipital gyrus, the medial frontal and superior gyrus and middle temporal gyrus (Brands et al., 2007; Zhang et al., 2014).

Recently, to assess changes in brain function, some studies have used degree centrality (DC) as a measure to investigate patterns of the functional connectome of the brain and analysis of functional connectivity (FC) in people with T2D (Kang et al., 2022; Liu et al., 2018). In relation to studies using the above-mentioned tests, Kang et al. (2022) showed reduced cortical thickness in middle-aged T2D patients compared to healthy, specifically in the right superior temporal and left transverse temporal gyri, as previously reported by Peng et al. (2015).

Other authors have focused on cognitive dysfunction and their relationship to changes in structure and hippocampal function (Liu et al., 2020; Milne et al., 2018). The hippocampus is a heterogeneous and complex structure and there is evidence that people with T2D have hippocampal atrophy (Hayashi et al., 2011; Moran et al., 2013). Some studies explored hippocampal laterality in these patients, with different subregions that differ anatomically and functionally. In this line, significantly reduced bilateral hippocampal volumes have been demonstrated in T2D (Li et al., 2021; Milne et al., 2018). It has been suggested that these structural changes may be related to difficulties in mnemonic processes, in particular, with impaired immediate memory (Liu et al., 2020).

To sum up, alterations in the functional connectivity of brain nuclei have been demonstrated in patients with T2D, which may translate into abnormal communication of information in the brain. In addition, the hypothesis explaining the abnormal brain function is that T2D accelerates the brain's ageing process, being an important cause of brain atrophy, hastening cognitive decline (Cui et al., 2017; Li et al., 2016; Van Elderen et al., 2010). Because of this, identifying changes in brain function could be crucial for understanding the risk of cognitive disorders in T2D.

Therefore, it is of great importance to carry out research that provides more data on factors that may reduce the difficulties presented by these patients. For example, it has been proposed that the risk of cognitive impairment in T2D may increase when experiencing stressful life events (Wang et al., 2023). In addition, exposure to external stressors has been linked to the onset of diabetes (Hackett and Steptoe, 2016). For that reason, it is important to understand how stress can affect cognitive performance, providing specific information on this subject in the following section.

Diabetes as a model of chronic stress

Stress is defined as a biological response resulting from extrinsic or intrinsic stimuli (Yaribeygi et al., 2017). Stress response can be understood at a physical, emotional or cognitive/behavioural level leading to serious consequences for the organism if sustained over the long term (Bao et al., 2008).

The current concept of stress has been developed from the contributions of Cannon and Selye. In 1929, Cannon first used the term “homeostasis” to describe the tendency to maintain the physiological stability in a dynamic equilibrium. The author indicated that a variety of threats to homeostasis elicited a sympathoadrenal response underlying to the “fight or flight” (Cannon, 1929). Selye discovered that the activation of the HPA axis appeared to be a common response to a variety of “noxious agents”, which initiate a reaction in the body called the “general adaptation syndrome” (GAS) (Selye, 1936; 1974). The GAS is characterized by three phases through which the body passes when stress is experienced: i) Alarm phase, when the body prepares itself for ‘fight or flight’; ii) Resistance phase in which stress confrontation takes place; and iii) Exhaustion phase, when stress remains for long periods of time, the body enters a stage of exhaustion, which can have a dangerous effect on the body (Selye, 1936). Thus, stress was an indeterminate response of the organism to any demand placed upon it (Selye, 1956; 1974).

But, over the last 50 years, neuroscience has expanded ideas about stress along many dimensions (McEwen and Akil, 2020). Biological responses to stress have been manifested not only in terms of reaching a negative state, but as a set of cumulative, continuous, and adaptive positive environmental assessment mechanisms that allows the individual to anticipate and cope with future challenges in order to enhance survival across the life course (McEwen and Akil, 2020). Furthermore, it has been shown that an inadequate or excessive level of stress may impair body composition, growth and development, as well as lead to pathological states. This confirms that uncontrollable stress is negative and can be physically and psychologically harmful (McEwen and Akil, 2020). In contrast, an optimal level of stress appears to be adequate for health (Chrousos, 2009).

The concept of homeostasis has been used with the primary meaning of maintaining internal stability through constancy. In 1988, Sterling and Eyer used the term “allostasis” defined as the process of actively maintaining and adapting stability through the action of several mediators, such as cortisol, to support adaptation. Later, to describe the stress process, the term “allostatic load” was first used by McEwen (McEwen, 2003; 2007). It is defined as the cost to the organism to maintain homeostasis when adapting to adverse physical or social situations (McEwen, 2000).

In this regard, it is important to note that there is evidence of an association between stress and T2D. In this sense, chronic stress seems to play an important role in the origin (Surwit et al., 1992) and development of T2D (Eriksson et al., 2013; Hackett and Steptoe, 2016; Heraclides et al., 2009). Therefore, chronic stress may influence the onset of the disease by increasing metabolic problems, producing hyperglycemia which can lead to insulin resistance, resulting in patients with T2D (Marcovecchio and Chiarelli, 2012; Sharma et al., 2022). In addition, Mooy et al. (2000) concluded that significant or stressful life events could lead to the onset of T2D in individuals with the disease predisposition, being this independent of physical exercise or educational level.

The following section provides information about the mechanisms underlying the stress response and its relationship with T2D.

Stress response

Two main components constitute the physiological response to stress: a fast response, mediated by the sympathetic-adrenomedullary (SAM) system, and a slow response mediated by the HPA axis (Chu et al., 2022). In response to stress signals, activation of the SAM system and the HPA axis as adaptive mechanisms allow the human body to maintain physiological stability. A lack of regulation of these systems can lead to metabolic abnormalities (Calandra-Buonaura et al., 2015).

In the face of a threat, the fight or flight response takes place, initiated by the SAM system, in connection with the HPA axis (Leonard and Myint, 2009). On the one hand, the activation of the SAM system leads to increased epinephrine (E) and norepinephrine (NE) secretion, starting in the medulla and spreading to the circulation. Elevated NE levels in the brain are also present as a consequence of increased NE secretion from sympathetic nerves. The β -adrenergic and α -adrenergic receptors of the central nervous system are those with which released E and NE interact. Once released, E and NE bind to specific membrane-bound receptors to initiate an intracellular cAMP signalling pathway. Activation of these receptors causes contraction of cardiac cells, leading to heart rate, increased blood pressure and glucose levels, increase in oxygen consumption, as well as behavioural activation (increased alertness, vigilance and cognition). On the other hand, when the HPA axis is activated, the secretion of corticotropin-releasing hormone (CRH) in the hypothalamus, followed by increased production and the secretion of adrenocorticotropin hormone (ACTH) from pituitary cells take place. This leads to glucocorticoid (GC) synthesis and secretion via the adrenal cortex. GCs then form part of a negative feedback loop of the HPA axis: GCs bind to glucocorticoid receptors (GR) in the paraventricular nucleus of the hypothalamus (PVN) and pituitary gland, inhibiting HPA axis activity through down-regulation of ACTH and CRH, and therefore reducing cortisol synthesis and release.

GCs bind to glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). GCs influence adaptive mechanisms and behavioural responses

to stress (Pfau and Russo, 2015; Russo et al., 2012), altering structural remodelling, synaptic plasticity and gene expression profiles. In addition, GCs modulate microglial function, maintaining a proinflammatory state (Frank et al., 2013; 2015). In the periphery, GCs induce apoptosis in T lymphocytes, monocytes and macrophages. In addition, they protect immune cells in the inflamed tissue by reducing the inflammatory response (Amsterdam et al., 2002).

Thus, the HPA axis is considered a complex neuroendocrine axis responsible for exerting the body's response to an emotional or physical stressor that may compromise the body's homeostatic balance (Habib et al., 2001). These variations at the endocrine level prevent possible negative consequences for the individual, and adapt to homeostasis risk (Leonard and Myint, 2009).

The sympathetic, and the parasympathetic branches are the components for the autonomic nervous system (ANS) that regulate the visceral functions (Sanvictores et al., 2023). The ANS, the HPA axis, and the immune system work together in achieving the hormonal and inflammatory stress response (McEwen et al., 2015; Ulrich-Lai and Herman, 2009). Thus, an acute stressor and the consequent response can constitute an adaptive mechanism of the endocrine system with protective effects. In contrast, sustained and repetitive stress may result in detrimental changes, leading to long-term alterations of the HPA axis and pathological conditions (De Kloet et al., 2005).

CRH coordinates autonomic, cognitive and behavioural responses to stress stimuli, complementing the endocrine responses of the HPA axis (De Kloet et al., 2005). CRH produces modifications in the sympathoadrenal system and the HPA axis, including activation of central and peripheral macrophages and producing pro-inflammatory agents, as well as an accumulation of abdominal fat and hypertension. Increased CRH production in the hypothalamus can sustain prolonged activation of the HPA axis. CRH also exerts an activating effect on the central sympathetic system by activating the locus coeruleus. The acute stress response is regulated by a feedback loop, slowing down the release of CRH and ACTH (Leonard and Myint, 2009).

In a situation of chronic stress, GC resistance is associated with a decrease in anti-inflammatory processes due to a maintained state of pro-inflammatory mediators (Cohen et al., 2012). In the stress response, a chronic activation of biological systems can lead to dysregulated physiological reactivity contributing to negative health outcomes with increased vulnerability to some disorders (McEwen and Wingfield, 2003) such as metabolic syndromes, insulin resistance and T2D (Black, 2006). In T2D, stress-related biology has been shown to be altered and multiple biological components such as increased inflammation, cortisol and dysregulation of the cardiovascular system may be present, reflecting chronic allostatic load (Hackett et al., 2017).

In sum, stressful circumstances have been associated with T2D. Therefore, it might be important to measure stress levels in these patients, providing more information on some of the biomarkers in the next section.

Stress response in T2D

To achieve accurate measurement of HPA-axis activity, cortisol levels can be reflected through saliva (Vining et al., 1983). This non-invasive measure allows samples to be collected outside the laboratory environment. By collecting them, basal cortisol levels as well as specific time points during the day can be assessed, thus eliminating the influence of laboratory stress. In addition, cortisol has been also measured employing acute laboratory stressors (Pulopulos et al., 2018). These biological samples are an attractive measure in research (Inder et al., 2012; Turpeinen and Hämäläinen, 2013), because they are easier to obtain and offer good control of the time of sampling (Golden et al., 2011).

Saliva samples have been used to study sAA and cortisol given its advantages when compared to other type of samples (Prasad et al. 2016). For example, compared to blood, one of the most important advantages is that recollecting saliva is minimally invasive (Tvarijonaviciute et al., 2020). Furthermore, in comparison to other non-invasive samples as urine and faeces, saliva is more convenient because the simplicity for storage and transport (Yoshizawa et al. 2013).

Regarding the sAA response in older T2D, the research is scarce. Only Siddiqui et al. (2015) used sAA biomarker in T2D participants, reporting a positive relationship between perceived stress and sAA levels. Therefore, more studies need to delve into the association between sAA levels and T2D.

In relation to the HPA axis response, an increased HPA axis response has been evidenced in individuals with T2D in neuroendocrine challenge studies. In this regard, Bruehl et al. (2007) showed an increase in CRH responsiveness. These results concluded an abnormality in the feedback sensitivity of the HPA axis, suggesting a possible predictor of T2D (Taheri et al., 2018). Reynolds et al. (2010) also reported elevated morning and evening cortisol levels in older people with T2D, and elevated ACTH and cortisol levels following repeated stress (Reynolds et al., 2001).

Regarding acute stress, some studies showed decreases in cortisol immediately after a mental stressor, analysing it as a disruption in stress-related biological systems (Carvalho et al., 2015; Steptoe et al., 2014), although Faulenbach et al. (2011) observed higher cortisol levels in T2D participants after exposure to a psychosocial acute stressor (Trier Social Stress Test, TSST). This test was also used by Monzer et al. (2022), who showed impaired ANS functioning, manifested by a slower HR recovery and attenuated HR increase in response to psychosocial stress. In this context, there seems to be a clear relationship between T2D and acute stress. For this reason, the stress response in T2D should be considered as a potential factor to be considered in the management of T2D.

The diurnal cortisol pattern in T2D

As already mentioned, salivary cortisol reflects stress-related changes in the HPA axis in both: as response to a stressor, as well as in basal conditions. Regarding the latter, the diurnal cortisol cycle is considered an indicator of HPA axis health, since it reflects the diurnal HPA axis functioning under normal or non-stress situations (Miller et al., 2007).

It is well known that the cortisol circadian rhythm shows a pattern characterized with a rapid rise in cortisol levels upon awakening (peaking at 30–45 min) and gradually declining throughout the day (Pruessner et al., 1997). A diurnal cortisol curve can be constructed by timed saliva collection and may include the following indexes: (i) Cortisol awakening response (CAR; the rise in cortisol from that occurs from 30 to 45 min after awakening); and (ii) the diurnal cortisol slope (DCS; the cortisol decline during the day, frequently calculated by the difference between awakening and evening cortisol (Adam et al., 2017).

Stress and cognitive function in T2D

Acute stress and memory performance in T2D

T2D is associated with high stress situations as a consequence of the demands and control to which a person with a chronic disease is subjected (Skinner et al., 2019). Episodes of acute stress affect the body (Juarez, 2020) through hormonal changes that cause body dysregulation (Hoogendijk et al., 2019). This can affect glycemic control in patients with T2D (Marcovecchio and Chiarelli, 2012). Along this line, episodes of hypoglycemia (Aung et al., 2012) and hyperglycemia (Cerasuolo and Izzo, 2017) have been associated with alterations in WM in the T2D population (Ryan et al. 2006). In addition, cross-sectional studies in older T2D individuals demonstrated that noradrenergic (NA) activation and GCs action affect brain activation in frontal region, during a WM task (Chen et al., 2014), in response to stress (Patel et al., 2000; Schoofs et al., 2008).

It is evident that stress affects the population in general, and T2D patients in particular, as well as that some cognitive processes, specifically WM, may be affected in the population with this disease. However, to our knowledge, no previous studies have investigated the involvement of acute stress on WM in patients with T2D.

Therefore, more research is needed to understand the psychobiological response to an acute stressor and its effect on cognitive performance in T2D individuals. This topic will be addressed in Chapter 3 of this thesis.

It is well known that stressful events, by means of the hormones and neurotransmitters secreted, affect the temporal and prefrontal brain, fundamental areas for information retrieval (Joëls and Baram, 2009). In the healthy aging population, the face of a chronic and/or acute stress causes altered cortisol response that may affect hippocampal-dependent memory (Lupien et al., 1997). In this line, Dos Santos et al. (2017) showed altered DM performance after an acute stressor in healthy older people. In T2D patients, worse DM performance has also been demonstrated, although the biological explanation still remains unanswered (Awad et al., 2004). To our knowledge, the impact of acute stress on DM in T2D has not been sufficiently investigated. Therefore, further research is needed to understand the involvement of stress in information retrieval in T2D patients. This topic will be addressed in Chapter 4 of this thesis.

Diurnal cortisol cycle and cognitive function in T2D

Normally, the cortisol circadian rhythm shows a pattern characterized by cortisol rising post-awakening, peaking at 30–45 min, known as C awakening response (CAR) and gradually declining over the following hours to reach the lower levels at the end of day. The difference in cortisol from morning to evening permits to calculate the Diurnal Cortisol Slope (DCS) (Adam et al., 2017). For studying the cortisol circadian rhythm, a diurnal cortisol curve is constructed by timed saliva collection. This curve includes the CAR and the DCS index previously mentioned, among others. An altered circadian cortisol secretion was reported in T2D, as compared with healthy individuals (Lederbogen et al., 2011). Specifically, a flattened CAR has been reported in T2D patients when compared with healthy individuals (Bruehl et al., 2009; Panagiotou et al., 2021; Lederbogen et al., 2011) although not always (Hackett et al., 2014).

Similarly, a blunted DCS has been found (Hackett et al., 2014; Lederbogen et al., 2011) whereas in other studies no differences with healthy controls were described (Bruehl et al., 2009).

Therefore, the results are heterogeneous in relation to the study of cortisol in patients with T2D. Furthermore, the relationship between cortisol and some cognitive domains has not been previously studied, being important to establish more clearly how T2D is related to HPA dysregulation and the relationship between cortisol and cognitive functions in this population. The relationship of CAR and DCS with cognitive performance in several cognitive domains will be presented in the fifth chapter of this thesis.

Chapter 2

Objectives and hypotheses

The present doctoral dissertation investigates whether activity of the HPA-axis and SNS are related to cognitive performance in older T2D people. Three studies with the following aims and hypotheses were performed:

Study 1: The main purpose of the first study was to characterise the psychobiological response (i.e., affect, sAA and salivary cortisol) of older men and women with T2D to an acute psychosocial stressor and its effects on WM performance, as no previous studies have investigated the effects of acute stress on WM performance in older T2D. Based on previous research performed in healthy people, we expected that participants in the stress condition would show increases in negative mood, decreases in positive mood and higher C and sAA levels than participants in the control condition. In addition, we expected an impairing effect of stress on WM performance according to the lower WM performance found in people with T2D.

Data and results obtained to respond to these research objectives are presented in Chapter 3.

Study 2: The aims of the second study were to investigate the psychobiological response (i.e., affect, sAA and salivary cortisol) to an acute psychosocial stress in older people with T2D in comparison with a group of age and sex matched healthy participants, and to explore the acute stress impact on declarative memory retrieval performance. According to previous studies in healthy population, we hypothesized that the psychobiological response will be higher in the stress condition and, specifically, the cortisol response of T2D participants will be more pronounced in comparison to healthy participants. Moreover, according to previous findings in healthy older people, we expected no effects of acute stressor on memory retrieval performance.

Data and results obtained to respond to these research objectives are presented in Chapter 4.

Study 3: The objectives of the third study were to investigate the diurnal cortisol cycle and the cognitive performance in T2D, and to analyse the relationships between two indexes of the diurnal cortisol cycle (i.e., CAR and DCS) and cognitive performance (i.e., DM, WM, and EF). Finally, we also wanted to explore the role of depression in the relationships among cortisol indexes and cognitive domains. To do this, in this study as in study 2, T2D patients were compared to matched healthy older people. Based on previous studies, we expected that T2D group will have a flatter CAR and DCS as well as worse cognitive performance in comparison to controls. In addition, for both T2D and healthy groups, we anticipated that a CAR of increased magnitude will be associated with poorer DM performance, but, at the same time, with better WM and EF performance. Moreover, we also hypothesized that a flatter DCS will be associated with lower DM. However, we had no a priori assumptions about the association between WM and EF with DCS in T2D population. Finally, we would expect that the greater depression found in T2D participants would increase the strength of the relationship between cortisol indexes and cognitive functioning.

Data and results obtained to respond to these research objectives are presented in Chapter 5.

Chapter 3

Study 1: Acute stress and working memory in type 2 diabetes¹

¹Main results of this chapter have been published in: Vallejo L, Zapater-Fajarí M, Montoliu T, Puig-Perez S, Nacher J, Hidalgo V and Salvador A (2021) No Effects of Acute Psychosocial Stress on Working Memory in Older People With Type 2 Diabetes. *Front. Psychol.* 11:596584

Introduction

Type 2 diabetes (T2D) has been considered a public health threat (Rucker et al., 2012) because of its growing prevalence (Exalto et al., 2013), particularly in the older population (Rucker et al., 2012). Disturbances in blood glucose (BG) homeostasis in these individuals can lead to transient or permanent alterations, such as decreased psychomotor speed and memory deficits (Yeung et al., 2009). In addition, learning also seems to be very sensitive to glycemic variations (Ryan and Geckle, 2000) and poorer metabolic control (Ryan et al., 2016), which are associated with the development of cognitive complications (Ryan et al., 2006) and a faster decline on memory measures compared to normo-glycemic people (Tuligenga et al., 2014). Furthermore, reduced frontal lobe/executive function has been found in the T2D population, including in certain memory domains such as working memory (WM) (Kim, 2019). Prefrontal cortex also contributes to other cognitive functions, such as reasoning, planning, and decision-making (Funahashi, 2015, 2017), which are essential for well-being and quality of life. Hence, the impact of T2D on these cognitive functions is a crucial topic that should be addressed.

In this sense, Ryan et al. (2006) reported that subjects with poor glycemic control had difficulty performing the WM tasks. Furthermore, WM impairments associated with episodes of both hypoglycemia (Aung et al., 2012) and hyperglycemia (Cerasuolo and Izzo, 2017) have been found in diagnosed T2D patients. However, Arvanitakis et al. (2006) concluded that T2D was not associated with worse WM performance.

There is a large body of evidence about the relationship between stress and T2D. In fact, chronic stress seems to play an important role in the etiology (Surwit et al., 1992) and development of T2D (Agardh et al., 2003; Heraclides et al., 2009; Eriksson et al., 2013; Hackett and Steptoe, 2016). In this line, Mommersteeg et al. (2012) showed that high psychological distress was a risk factor for the development of diabetes in an 18-year follow-up study. Furthermore, a prospective 35-year follow-up study reported that high perceived stress in adulthood increased the risk of T2D (Novak et al., 2012). It has been well

established that the exposure to stress is associated with the activation of both the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. Consequently, large amounts of alpha-amylase (an indirect marker of SNS) and cortisol (C), the main human glucocorticoid, are secreted. The chronic activation of these two systems produces an increase in BG levels, contributing to T2D development (Boaz et al., 2013; Lundqvist et al., 2019).

Regarding acute stress, higher glucose and C levels were reported in T2D patients after exposure to a psychosocial stressor (i.e., Trier Social Stress Test, TSST), in comparison with a control session (Faulenbach et al., 2011). However, significant C decreases immediately after a mental stressor have also been found and interpreted as a disruption in stress-related biological systems (Steptoe et al., 2014; Carvalho et al., 2015). To the best of our knowledge, no previous study has investigated the salivary alpha-amylase (sAA) response to an acute stress in T2D patients. Only one study has analyzed the relationships between sAA levels and perceived stress in T2D patients (Siddiqui et al., 2015), and it reported a positive relationship between them.

It has been well established that acute stress affects memory processes (Lindau et al., 2016; Hidalgo et al., 2019), given that there is an overlap of the brain structures involved in the stress response and memory processes. Specifically, the prefrontal cortex and the hippocampus, brain areas with a high density of glucocorticoid receptors, are involved in WM (Galloway et al., 2008; Kumar et al., 2016). It is important to note that most of the studies investigating stress-induced cortisol effects on WM have been carried out in healthy young people (for a review see Hidalgo et al., 2019), with only one in healthy older people (Pulopulos et al., 2015). In this study, the authors failed to find acute stress effects on WM performance, even though participants showed C and sAA responses to the TSST.

In addition, there is evidence of an association between cognitive performance in T2D and hippocampal integrity (Jones et al., 2014). This structure is especially sensitive to the effects of hypoglycemia (Marks and Rose, 1965) and higher BG levels, leading to hippocampus and amygdala atrophy (Cherbuin et al.,

2012). Hippocampal atrophy was found in individuals with T2D, which could correlate with impairments in immediate memory (Gold et al., 2007). However, no previous studies have investigated the acute effects of stress on WM in T2D older people in spite of, as noted above, T2D patients show deficits in WM (for reviews see: Palta et al., 2014; Pelimanni and Jehkonen, 2018; Kim, 2019).

Hence, the aim of the present study was to assess the psychobiological (i.e., mood, sAA, and C) response to an acute stressor (i.e., TSST) and its effect on WM performance in diagnosed and medically treated older people with T2D. We expected that participants in the stress condition would show increases in negative mood and decreases in positive mood (Almela et al., 2011; Hidalgo et al., 2020) and higher C and sAA levels (Almela et al., 2011; Pulpulos et al., 2015) than participants in the control condition. In spite of the lack of significant findings in healthy older people (Pulpulos et al., 2015), based on worse WM performance in people with T2D (Gorniak et al., 2018), we expected that acute stress would impair WM performance.

Material and Methods

Participants

The final sample was composed of 50 participants from 52 to 77 years old, 24 men and 26 women, with a medical diagnosis of T2D. They were randomly assigned to two conditions in a counter-balanced way: 24 to the stress condition (12 men and 12 women) and 26 to the control condition (12 men and 14 women). No gender differences were found between the two conditions ($\chi^2 = 0.074$, $p = 0.786$) (see Table III.1 for sample characteristics).

Table III.1. Descriptive statistics (mean $\pm$ SEM) for total sample and both two conditions.

	Total sample (N = 50)	Stress Condition (N = 24)	Control Condition (N = 26)	t , χ^2
Age (years)	65.64 (0.724)	66.54 (0.936)	64.81 (1.083)	$t = 1.202$, $p = 0.235$
BMI (kg/m ²)	28.77 (0.725)	28.411 (0.993)	29.094 (1.059)	$t = -0.467$, $p = 0.643$
SES	5.233 (0.216)	5.348 (0.324)	5.114 (0.290)	$t = 0.536$, $p = 0.594$
Time diagnosed (years)	11.02 (1.114)	10.75 (1.592)	11.33 (1.583)	$t = -0.259$, $p = 0.797$
Educational level (%)				$\chi^2 = 3.240$, $p = 0.356$
No studies	6.1	0	11.5	
Basic studies	34.7	39.1	30.8	
High school	28.6	26.1	30.8	
College or higher	30.6	34.8	26.9	
Marital status (%)				$\chi^2 = 3.265$, $p =$ 0.353
Single	18.4	21.8	15.4	
Married	65.3	65.2	65.4	
Divorced	10.2	13	7.7	

Widowed	6.1	0	11.5
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%, percentages; BMI, body mass index; SES, subjective socioeconomic status-scale (Adleret al., 2000; from 1: lowest to 10: highest level).

Participants were recruited from hospitals and medical clinics and referred by their doctors; all of them were being medically treated and controlled during the period of the research. All volunteers were interviewed by telephone in order to find out if they met the exclusion criteria, which were: smoking more than 10 cigarettes per day, abuse of alcohol or other drugs of abuse, presence of severe cardiovascular disorder, psychiatric or neurological disorder, visual or hearing impairments, having been under general anesthesia in the past year, the presence of a stressful life event in the past year, and consuming drugs related to cognitive or emotional functions or psychotropic substances. As T2D patients, they were medicated as follows: 54.5% oral antidiabetic, 34.1% oral and injectable, and 11.4% only injectable. Additionally, volunteers who used other medications that could influence hormonal levels (glucocorticoids, beta-blockers, antidepressants, asthma medication, or thyroid therapies) were excluded from the study.

All participants received verbal and written information about the study and signed an informed consent form. The study was carried out in accordance with the Declaration of Helsinki, and the Ethics Research Committee of the University approved the protocol.

Procedure

Participants attended an individual session held between 16:00 and 18:00 in the Laboratory of Social Cognitive Neuroscience at the University of Valencia. Before the session, the experimenter first checked whether each participant had followed the instructions prior to participation: refrain from heavy physical activity from the evening before the session, sleep as long as usual, and not consume alcohol since the night before the session. Additionally, participants were instructed to drink only water and not eat, smoke, take any stimulants (e.g., coffee, cola, tea, chocolate), or brush their teeth at least 2 h prior to the session.

Stress Condition

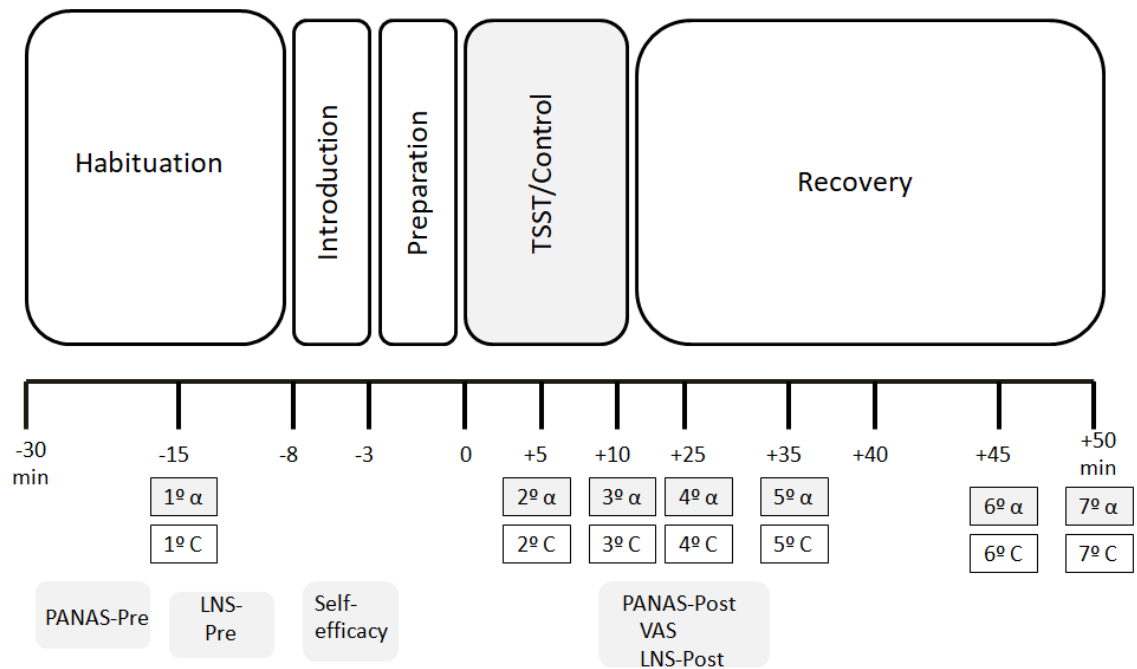
To produce stress, we employed the TSST (Kirschbaum et al., 1993). This task consisted of 5 min of free speech (simulated job interview) and 5 min of an arithmetic task. Participants stood in front of a committee with a video camera and a microphone clearly visible. Before the TSST, participants completed several items to assess their perceived self-efficacy and the PANAS (pre-task PANAS) to obtain the baseline measure for mood. In addition, WM was evaluated with Letters and Number Sequencing (pre-task LNS). Immediately after the TSST, participants filled in some items about the task (Situational Appraisal), the PANAS (post-task PANAS), and the LNS (post-task LNS).

Control Condition

This condition was similar to the stress condition in terms of mental workload and global physical activity, but it lacked the main components that could provoke stress, such as evaluative threat and uncontrollability (Dickerson and Kemeny, 2004). The control task consisted of 5 min of free speech about a recent non-emotional experience, followed by an arithmetic task consisting of 5 min of counting by 5 aloud. This control task has been used in previous studies (Pulopulos et al., 2013, 2019; Puig-Perez et al., 2015).

During each session, we collected seven saliva samples to measure sAA and C levels. Specifically, the first saliva sample was collected prior to the stress/control task at habituation (–15 min pre-stress). The rest of the saliva samples were taken during the stress/control task (+5 min), after the TSST/Control task (+10 min post-stress), three at 10 min periods after the task (+25, +35, and +45 min), and finally at +50 min post-stress. In addition, two capillary blood samples were taken at habituation (–20 min) and in the recovery period (+55 min) to measure glucose concentrations. Both conditions followed the same time schedule for sample collection, phase durations, and questionnaires administered, and they only differed on the task (Stress vs. Control) (see Figure III.1).

Figure III.1. Timeline of the TSST (S) and control (C) conditions. Salivary cortisol samples = 1^o C, 2^o C, 3^o C, 4^o C, 5^o C, 6^o C, 7^o C. Salivary alpha-amylase samples = 1^o α, 2^o α, 3^o α, 4^o α, 5^o α, 6^o α, 7^o α. Time of collection of saliva samples (-15, +5, +10, +25, +35, +45, +50). TSST = Trier Social Stress Test. PANAS=Positive and negative affect; LNS= Letter-number sequencing; VAS = Situational appraisal.



Psychological Assessment

Self-Efficacy

Participants' self-efficacy, understood as beliefs about one's capacity to organize and carry out actions (Bandura, 1997), was evaluated with three items (van der Meij et al., 2010; Villada et al., 2017). These items were formulated to measure capacity ("How capable are you of successfully performing the task?"), self-confidence ("How confident are you that you will successfully perform the task?"), and importance of successfully performing the task ("How important is it to you to perform this task successfully?"). Participants had to respond on a Likert scale ranging from "1 = not at all" to "100 = totally."

Situational Appraisal

Several items were used to measure the participants' situational appraisal (Gonzalez-Bono et al., 2002), focusing on the following aspects: frustration, motivation, amount of perceived stress, difficulty of the task, assessment of their own performance, expected outcome of the evaluation, and perception of the effort required (e.g., How much effort did the task require?). Each item was rated on a 5-point Likert scale (not at all = 1 to extremely = 5).

Mood

The Spanish version (Sandín et al., 1999) of the Positive and Negative Affect Scale (PANAS) (Watson et al., 1988) was employed. This 20-item questionnaire assesses mood in two dimensions: positive affect (PA: interested, excited, strong, enthusiastic, etc.) and negative affect (NA: distressed, upset, guilty, scared, etc.), with 10 items measuring each state. The participants were asked to complete the questionnaire based on how they felt at that particular moment. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). In the current study, Cronbach alpha coefficients were 0.91 and 0.87 for PA and NA, respectively.

Working Memory Task

The Letter–Number Sequencing (LNS) task from the Wechsler Memory Scale III (Wechsler, 1997) was used to assess WM performance. This test requires participants to listen to sequences of alternating digits (ranging from 0 to 9) and letters (from A to Z) of increasing lengths. Then, they have to repeat the digits and letters from each sequence, beginning with the digits in numerical order and then the letters in alphabetical order. The length of the sequences increased from two to eight items, and participants were allowed three attempts to solve each sequence length. One point was assigned for each correct attempt, and the task ended when the participant had failed the three attempts for the same sequence length. The raw scores range from 0 to 21.

The longest score, the last item, has a maximum of 7. We calculated three outcomes from this test: (i) LNS pre-task: Total number of correctly recalled attempts pre-task; (ii) LNS post-task: Total number of correctly recalled attempts post-task; and (iii) Retention Rate: percentage of the score after the TSST/control task compared to the total score before the task ($\text{WM post-task}/\text{WM pre-task} \times 100$).

Biochemical Analyses

Salivary samples were collected using salivettes (Sarstedt, Nümbrecht, Germany). Participants were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all the salivary glands. The samples were kept in the refrigerator until they were centrifuged at 3,000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was stored at -80°C until the analyses. For each subject, all C and sAA samples were analyzed in the same trial. C levels were analyzed using the commercial enzymeimmunoassay kit from Salimetrics (Newmarket, United Kingdom). The sensitivity of the assay was $<0.007\text{ }\mu\text{g/dL}$, and the intra- and inter-assay coefficients of variation were all below 10%. C levels were expressed in nmol/L.

The sAA concentration was measured by using an enzyme kinetic method with the commercial salivary sAA assay kit from Salimetrics (United States). Assay sensitivity was 0.4 U/mL . Inter- and intra-assay variation coefficients were all below 10%. sAA concentrations were expressed in U/mL.

Two capillary blood samples taken at habituation (-20 min) and recovery period ($+55$) were employed to measure glucose concentration using a glucose monitoring system (Onetouch ultraeasy life Scan Europe 6300 Zug, Switzerland, AW 0639870).

Statistical Analyses and Data Management

Student's *t*-test was used to analyze socio-demographic differences between stress and control participants, whereas differences in educational level and marital status were analyzed with Chi-square tests.

To investigate the effects of stress on self-efficacy and situational appraisal, multivariate analyses of variance (MANOVA) were performed with each self-efficacy (i.e., capacity, self-confidence, and motivation) and situational appraisal (i.e., effort required, frustration, own performance, stress perceived, difficulty, importance of doing the task, and expected outcome) item as dependent variable and Condition as independent variable.

In order to investigate the effects of stress on mood, glucose levels, and WM performance (LNS), ANOVAs for repeated measures were performed, with Condition as a between-subject factor and Time (pre vs. post) as a within-subject factor.

Because C and sAA values did not show a normal distribution, they were log transformed. ANOVAs for repeated measures with Condition (stress vs. control) as a within-subject factor and Time (-15, +5, +15, +25, +35, +45, +50 min) as a between-subject factor were performed to assess the C and sAA levels. Partial eta squared (η^2) is reported as a measure of effect sizes for ANOVAs (Cohen, 1973).

Finally, to assess whether the psychobiology response to the stressor was related to WM performance, two regression analyses were performed only for the participants in the stress condition. In one regression we included in Step 1 $C_{reactivity}$ (maximum C levels after the stressful task minus baseline period) and $sAA_{reactivity}$ (maximum value on the stressful task minus baseline period) and in Step 2 the interaction between $C_{reactivity}$ and $sAA_{reactivity}$. In the second regression, we included in Step 1 the positive affect reactivity (PAreactivity) and negative affect reactivity (NAreactivity), that is, the positive or negative affect post task minus positive or negative affect pre task, respectively.

Two outliers in the mood data, two outliers in the glucose data, one outlier in the cortisol data, and two outliers in the sAA data were excluded from the analyses because their values differed by more than 3 *SD*. from the mean. In addition, one woman was removed because her scores on the WM retention rate differed by more than 3 *SD*.

Greenhouse–Geisser was used when the requirement of sphericity was violated in the ANOVA for repeated measures. *Post hoc* planned comparisons were performed using Bonferroni adjustments for the *p*-values. Analyses were carried out using SPSS 25.0. All *p* values reported are two-tailed. The level of significance was fixed as <0.05. For easy interpretation, the values in the figures represent raw values.

Results

Preliminary Analyses

No condition effects were found on age ($p = 0.235$), education level ($p = 0.356$), SES ($p = 0.594$), BMI ($p = 0.643$), marital status ($p = 0.353$), or years of diagnosis ($p = 0.797$).

Self-Efficacy

The MANOVA showed significant differences between conditions in capacity [$F_{(1, 47)} = 10.144, p = 0.003, \eta^2_p = 0.178$] and self-confidence [$F_{(1, 47)} = 4.215, p = 0.046, \eta^2_p = 0.082$]; participants in the stress condition reported less ability and less confidence about performing the task successfully than those in the control condition. No significant differences in motivation [$F_{(1, 47)} = 0.748, p = 0.391, \eta^2_p = 0.016$] were observed.

Situational Appraisal

The MANOVA showed that the stress task was perceived as more stressful [$F_{(1, 44)} = 7.796, p = 0.008, \eta^2_p = 0.151$], frustrating [$F_{(1, 44)} = 17.438, p < 0.001, \eta^2_p = 0.284$], difficult [$F_{(1, 44)} = 65.838, p < 0.001, \eta^2_p = 0.599$], and requiring more effort [$F_{(1, 44)} = 20.777, p < 0.001, \eta^2_p = 0.321$] than the control task. In contrast, participants in the control condition thought they performed the task better [$F_{(1, 44)} = 36.529, p < 0.001, \eta^2_p = 0.454$] and had better results [$F_{(1, 44)} = 35.675, p < 0.001, \eta^2_p = 0.448$] than those in the stress condition. No significant differences in the importance of doing the task were observed between conditions [$F_{(1, 44)} = 0.390, p = 0.536, \eta^2_p = 0.009$].

Glucose Levels

Regarding the glucose levels, the ANOVA for repeated measures showed a main effect of Time [$F_{(1, 46)} = 5.857, p = 0.020, \eta^2_p = 0.113$]. However, no effects of Condition [$F_{(1, 46)} = 0.202, p = 0.655, \eta^2_p = 0.004$] or the Time $\times$ Condition interaction [$F_{(1, 46)} = 0.537, p = 0.468, \eta^2_p = 0.012$] were found. In both conditions, participants showed significantly lower glucose levels after the task than before it (Pre task: $M = 153.36, SEM = 6.86$; Post task: $M = 142.76, SEM = 7.52$).

Psychobiological Stress Response

Positive and Negative Affect

The ANOVA for repeated measures did not show effects of Condition [$F_{(1, 46)} = 0.116, p = 0.735, \eta^2_p = 0.003$] on positive affect (PA). Although, the Time factor failed to reach significance [$F_{(1, 46)} = 3.972, p = 0.052, \eta^2_p = 0.079$], the Time $\times$ Condition interaction was significant [$F_{(1, 46)} = 6.447, p = 0.015, \eta^2_p = 0.123$]. *Post hoc* analyses showed that, overall, participants presented less positive affect after the stress task than before it. No differences were found between conditions before and after the task. However, participants in the stress condition reported significantly less PA after the stress task than before it ($p = 0.003$). No differences were observed in the control condition ($p = 0.695$) (Table III.2).

Table III.2. Mean $\pm$ SEM for mood changes, for total sample and both two conditions

	Time	Total sample	Stress condition	Control condition	<i>p</i>
Positive affect	Pre stress	31.195 (.977)	31.870 (1.410)	30.520 (1.353)	.493
	Post stress	29.589 (1.210)	28.217 (1.746)	30.960 (1.675)	.263
Negative affect	Pre stress	16.631 (.440)	16.636 (.635)	16.625 (.608)	.990
	Post stress	17.634 (.694)	19.227 (1.002)	16.042 (.960)	.027

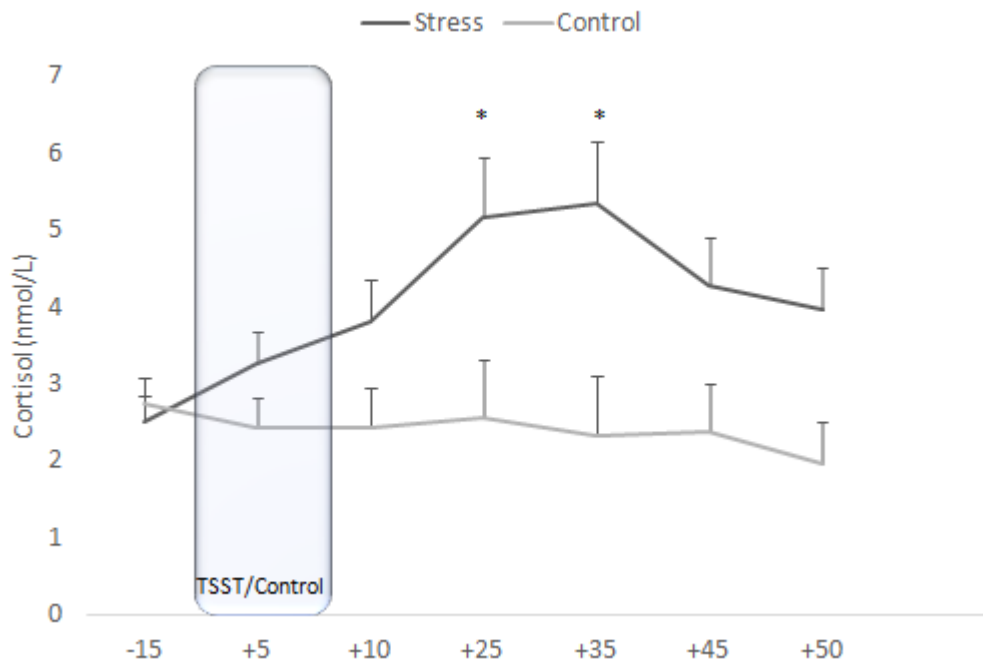
Regarding negative affect (NA), there were no significant effects of Time [$F_{(1, 44)} = 2.033, p = 0.161, \eta^2_p = 0.44$] or Condition [$F_{(1, 44)} = 2.911, p = 0.091, \eta^2_p = 0.064$], but the Time $\times$ Condition interaction was significant [$F_{(1, 44)} = 5.083, p = 0.029, \eta^2_p = 0.104$]. *Post hoc* analysis showed no differences between conditions

before the task. After the task, participants in the stress condition showed higher NA than participants in the control condition. Moreover, participants in the stress condition reported higher NA after the stress task than before it ($p = 0.014$). No differences were observed in the control condition ($p = 0.552$).

Cortisol

The ANOVA for repeated measures did not show an effect of Condition [$F_{(1, 43)} = 2.850, p = 0.099, \eta^2_p = 0.062$], although Time [$F_{(2, 101, 90.328)} = 3.167, p = 0.044, \eta^2_p = 0.069$] and the Time x Condition interaction were significant [$F_{(2, 101, 90.328)} = 6.807, p = 0.001, \eta^2_p = 0.137$]. *Post hoc* analyses showed that C levels were significantly higher in the stress condition than in the control condition +25 ($p = 0.026$), +35 ($p = 0.019$), and +50 ($p = 0.048$) min after the onset of the task. In addition, in the stress condition, there were no significant differences between C levels before and during the stress task (–15 vs. +5 samples: $p = 0.147$). However, C levels increased significantly from +10 min to +25 min ($p = 0.003$). After peaking at +35, cortisol levels started to decrease (+35 vs. +50 samples: $p = 0.032$) until finally reaching baseline levels (+50 vs. –15 samples: $p > 0.99$). In the control condition, no significant differences were observed (all $p > 0.147$) (Figure III.2).

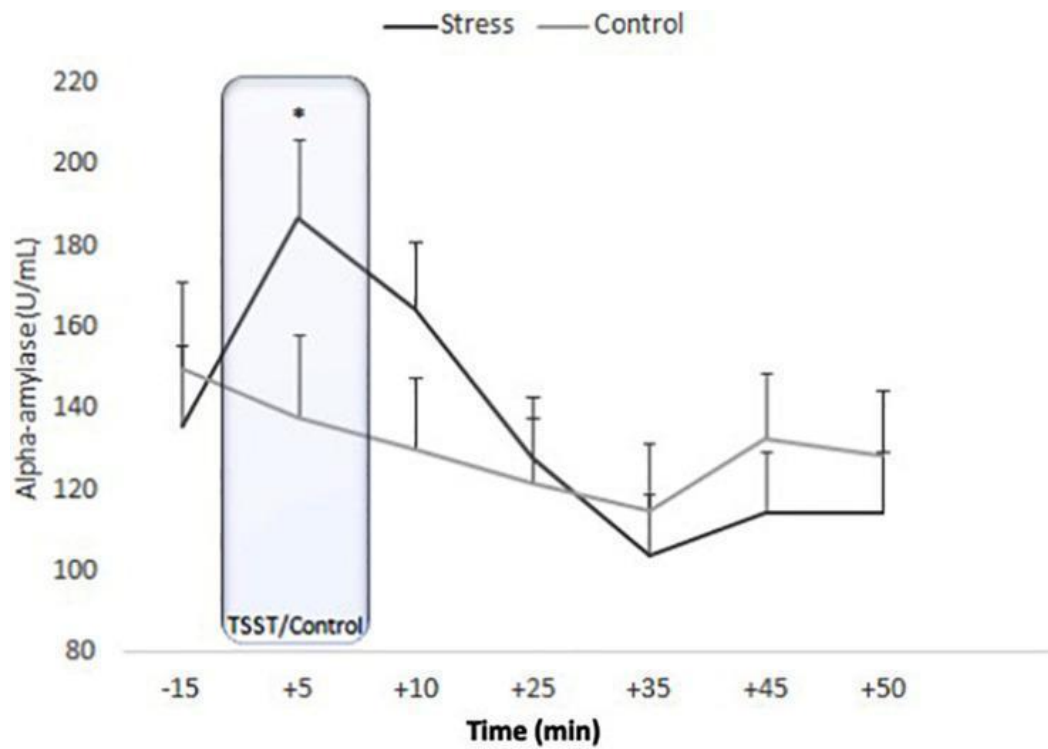
Figure III.2. Means of salivary cortisol concentrations for stress and control condition. Error bars represent standard error of mean (* $p < 0.05$).



sAA

ANOVA for repeated measures showed an effect of Time [$F_{(4.379, 170.765)} = 11.900, p < 0.001, \eta^2_p = 0.234$] and the Time x Condition interaction [$F_{(4.379, 170.765)} = 5.171, p < 0.001, \eta^2_p = 0.117$]. No effects of Condition were observed [$F_{(1, 39)} = 0.069, p = 0.795, \eta^2_p = 0.002$]. In the stress group, sAA levels started to increase at -15 min and were significantly higher at +5 min ($p = 0.002$). After peaking at +5 min, sAA levels significantly decreased (+5 vs. +35 samples: $p < 0.001$) and then remained stable (+35 vs. +50 samples; $p > 0.99$). In the control condition, no significant differences were observed (all $p > 0.137$) (Figure III.3).

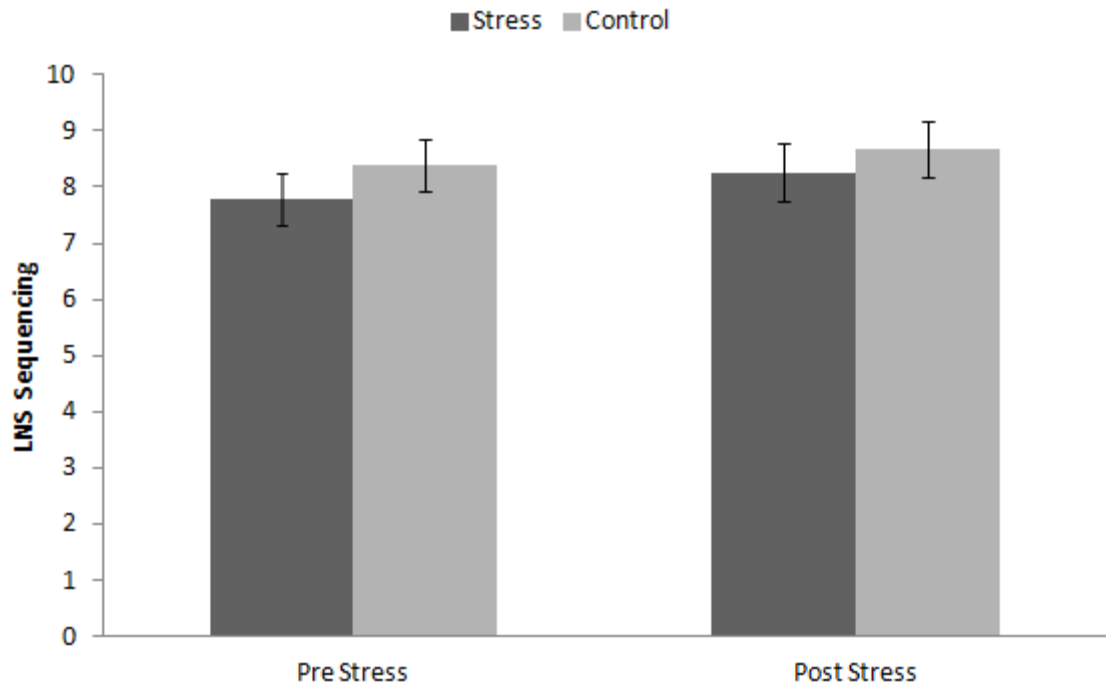
Figure III.3. Means of salivary alpha-amylase concentrations for stress and control conditions. Error bars represent standard error of mean (* $p < 0.05$).



Working Memory Performance

The ANOVA for repeated measures did not show significant effects of Time [$F_{(1, 46)} = 1.946, p = 0.170, \eta^2_p = 0.041$], Condition [$F_{(1, 46)} = 0.657, p = 0.422, \eta^2_p = 0.014$], or the Time $\times$ Condition interaction [$F_{(1, 46)} = 0.133, p = 0.717, \eta^2_p = 0.003$]. The performance in both groups was similar (Figure III.4).

Figure III.4. Performance on Letter-Number sequencing (LNS) for stress and control conditions before and after the task.



Relationship Between the Psychobiological Response and WM Performance

In the stress condition, the WM retention rate was not related to PAreactivity ($B = -0.367$, $p = 0.108$), NAreactivity ($B = 0.194$; $p = 0.383$), Creactivity ($B = 0.055$, $p = 0.831$), or sAAreactivity ($B = 0.038$, $p = 0.882$). However, the interaction term Creactivity $\times$ sAAreactivity was negatively associated with the WM retention rate ($B = -0.549$, $p = 0.026$). Thus, participants in the stress condition who showed higher C and sAA responses to the TSST had lower WM performance.

Discussion

The main purpose of the present study was to characterize the response of older men and women with T2D to an acute psychosocial stressor (i.e., TSST) and its effects on WM performance. As we expected, the TSST provoked significant mood changes and C and sAA responses in participants who were exposed to it, compared to those in the control condition. In addition, although stress did not affect WM performance, the WM retention rate was negatively related to the interaction between the C and sAA responses to stress.

Our results confirm that a psychosocial stressor (TSST) was able to produce a “typical” or characteristic psychobiological response in medically treated T2D patients. At the subjective level, the TSST was perceived as more stressful, frustrating, difficult, and requiring more effort than the control task, and the participants in the stress condition perceived that they had less ability to perform the task successfully. However, both stress and control participants showed a similar motivation to perform the task because they gave the same importance to their performance. Furthermore, the TSST elicited mood changes characterized by decreases in positive mood and increases in negative mood. This pattern confirms that this laboratory psychosocial stressor is able to generate a similar mood response in T2D patients to the one reported for healthy older adults (Hidalgo et al., 2020).

The TSST also provoked a clear C response in T2D patients. This result is consistent with a previous study (Faulenbach et al., 2011) reporting that, although C did not increase right after the stress task, the increase was detectable 30 min after the TSST in T2D patients. It is worth mentioning that in Faulenbach et al.’s study, T2D patients were subjected to the stress and control conditions in two different states (fasting or postprandial state). C increases were found in both states, although they were slightly greater in the fasting state. In our study, participants were in a short fasting state (1–2 h fasting period), and they showed significant reductions in their glucose levels throughout the session, regardless of the condition. Faulenbach et al. (2011) reported stable glucose concentrations in the TSST condition and slight reductions in the control

condition in the fasting state (a 10 h overnight fasting period, including stopping medication); in the postprandial state, they found higher glucose levels in the stress session than in the control session. In our case, after exposure to the TSST, participants in both conditions showed glucose decreases (4.52%) fairly similar to those in non-T2D groups (5.6%) assessed in our lab in other studies (unpublished data). It is worth noting that participants in this study were periodically supervised by their doctors and maintained adequate metabolic control.

In addition, in our study, participants showed a sAA response to the TSST. This finding is consistent with results found in healthy older people (Almela et al., 2011; Pulopulos et al., 2015). However, Strahler et al. (2010) found an attenuated sAA response to TSST in this age group. To the best of our knowledge, the current study is the first one to investigate the sAA response to an acute psychosocial stressor in older people with T2D. As in healthy older people, our results support the idea that the TSST also provokes a greater sympathetic-adrenal-medullary system response than the control task in older T2D subjects.

Until now, only a few studies have been carried out on sAA, with heterogeneous results in terms of differences between T2D and healthy people (Panchbhai et al., 2010; Shankaraiah and Reddy, 2011). We found higher levels of sAA in older adults with T2D undergoing acute psychosocial stress, compared to older subjects with T2D in a control condition. Our results are in line with those described in healthy older people (Pulopulos et al., 2015) and extend to T2D patients. In addition, they provide new information about the acute sAA response in T2D patients, and they confirm the relationship previously reported between sAA and perceived stress assessed by the Perceived Stress Scale (Siddiqui et al., 2015).

We did not find a significant effect of the TSST on WM performance or a relationship between the C response and WM performance. Our findings agree with other studies on both C administration (Wolf et al., 2001; Porter et al., 2002; Yehuda et al., 2007) and a stress-induced C response (Pulopulos et al., 2015) in healthy older people. Despite this, these results contrast with the conclusions observed in most studies with young male populations exposed to the TSST

(Luethi et al., 2009; Engert et al., 2011) and the cold-pressor test (Schoofs et al., 2009; Becker and Rohleder, 2019). The lack of stress effects on WM performance in our sample of older people with T2D extends previous findings found in healthy older people and supports the idea that older people may be less sensitive to the effects of stress on memory than young people, as we previously reported for WM (Pulopulos et al., 2015) and declarative memory (Pulopulos et al., 2013; Hidalgo et al., 2014, 2015) performance. This lack of stress effects on WM performance could be due to an age-related dysregulation of HPA-axis activity (Mizoguchi et al., 2009) and functional changes in the amygdala and hippocampus (Mather, 2006; Murty et al., 2009; St. Jacques et al., 2009) in older people. However, this lack of acute stress effects on WM in older people with T2D should be interpreted with caution, given that a healthy control older group was not included in the present study.

Although we failed to find a stress effect on WM performance, the interaction term between the C and sAA reactivities to the stressor was negatively associated with WM performance. Thus, participants who responded to stress with higher cortisol and sAA levels had worse WM performance. It has been well established that WM depends on prefrontal cortex functioning (Galloway et al., 2008), and that this brain area is affected by the glucocorticoid action and NA activation in response to stress (Patel et al., 2000; Schoofs et al., 2008). Hence, it is conceivable that this type of memory would be affected by the activation of both stress systems; therefore, the WM performance could be related not only to the HPA-axis or SNS action separately, but also to the interaction between these two systems. To the best of our knowledge, this association has not been previously reported in patients with T2D. Future studies are needed to support this association.

The present study has some limitations. To avoid as many confounding factors as possible, we were conservative and selected a homogeneous T2D sample in terms of age, sex, and other relevant sociodemographic characteristics. Consequently, the number of participants included was limited, which meant that we could not study possible gender differences in the acute stress effects on WM in older people with T2D.

Therefore, future studies should include bigger sample sizes and study the role of gender in this clinical population. In addition, although this study has a control group with T2D patients who did not undergo stress, further studies would benefit by also including other control group with healthy people. In spite of these limitations, the current study makes it possible to advance the knowledge about the characteristics of the acute response to psychosocial stress in medically treated T2D. In fact, our results suggest a psychobiological response similar to the one found in healthy older people, and different from that of young people. This similitude extends to the stress effects on WM for this age group. WM has great relevance in older people, and particularly in the T2D population, because many therapeutic actions are based on information that is necessary for a high degree of patient self-management.

Conclusions

In sum, this study provides interesting findings about the psychobiological response to acute psychosocial stress in older T2D men and women. The results provide empirical evidence about the cognitive and physiological response in older people with T2D with adequate medical supervision.

Chapter 4

Study 2: Acute stress on memory retrieval in older people with type 2 diabetes

Introduction

Type 2 diabetes (T2D), has emerged as a significant global health concern, affecting over 400 million individuals worldwide (International Diabetes Federation, 2019). Characterized by insulin resistance and impaired glucose metabolism, T2D is associated with a range of complications, including cardiovascular disease, neuropathy, and cognitive decline (Zheng et al., 2018). Recent research has begun to explore the intricate relationship between T2D and psychological factors, particularly stress. Chronic stress has been shown to influence metabolic processes, potentially exacerbating insulin resistance and dysregulation of glucose levels (Chrousos, 2009; McEwen, 2006). Furthermore, individuals with T2D often experience heightened stress due to the demands of managing their condition, creating a bidirectional relationship that may further complicate their health outcomes (Gonzalez et al., 2016). Understanding the interplay between T2D and stress is crucial for developing effective interventions aimed at improving both psychological well-being and metabolic control.

Despite the evidence about diabetes-stress link, less is known about the mechanisms linking psychological stress and T2D. The exposition to stress leads to, among other changes, increases in alpha-amylase and cortisol as consequence of the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis activation, respectively. In this sense, few studies investigate the psychobiological response (i.e., alpha-amylase and cortisol) to acute stress in T2D patients. Regarding alpha-amylase, only two previous studies investigated the link between stress and salivary alpha-amylase (sAA) levels (Siddiqui et al., 2015; Vallejo et al., 2021). Specifically, a positive association between perceived stress and sAA levels (Siddiqui et al., 2015), and sAA response to an acute stressor (Vallejo et al., 2021) have been reported. It is important to note that the latter study did not include a healthy control group, so, it seems clear the need to address the sAA response to acute stress compared with a healthy control group in order to know adequately the reactivity of T2D patients to acute stress in terms of sAA levels.

To the best of our knowledge, few studies have considered the cortisol response to acute stress of diabetic patients with mixed results. Thus, both strong cortisol response to the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) (Buckert et al., 2022; Faulenbach et al., 2013; Vallejo et al., 2021) and blunted cortisol response to a mild mental stressor (Steptoe et al., 2014) have been found. Methodological differences could help to understand these inconclusive results. In addition to the stressor used (i.e., TSST vs. mental stressor), the type of biological sample used to assess cortisol levels (blood: Buckert et al., 2022; saliva: Faulenbach et al., 2013; Steptoe et al., 2014; and Vallejo et al., 2021), or the inclusion of a healthy control group (Buckert et al., 2022; Steptoe et al., 2014) or not including it (Faulenbach et al., 2013; Vallejo et al., 2021) were different across studies. Taking together, the study of the psychobiological response to an acute psychosocial stressor in diabetic patients is a crucial topic that needs further research.

It is well known that stress affects cognitive function, especially memory performance (for a review see: Hidalgo et al., 2019). In fact, the control of both the stress response and memory function shares the same brain areas (i.e., prefrontal cortex, hippocampus and amygdala). Thus, stress hormones (i.e. catecholamines and cortisol) impact memory through different pathways, binding to glucocorticoid receptors widely located to these brain structures and/or with the noradrenergic activation of the amygdala (McGaugh and Roozendaal, 2022). These biological pathways could be the key to finding cortisol effects on hippocampus-dependent memory performance being declarative memory the type of memory mostly studied in the stress effects on memory function (Hidalgo et al., 2019). Interestingly, in T2D patients there is an increased risk of cognitive dysfunction (Casagrande et al., 2021; Dao et al., 2022; Umegaki et al., 2013) being the risk of accelerated cognitive impairment 1.5-2.0 times higher in them in comparison to healthy participants (Biessels and Despa, 2018; Xiong et al., 2020). Multiple cognitive domains are compromised in diabetes, including declarative memory (Geijselaers et al., 2015; Li et al., 2021; Ravona-Springer et al., 2014), maybe due to the hippocampus is rich in insulin receptors (Pomytkin et al., 2018).

For example, an impairing on immediate and delayed verbal recall (Debling et al., 2006; Grodstein et al., 2001; Yau et al., 2014) have been reported, but this effect was no longer found on short and long-term memory after controlling for vascular risk factors (Xie et al., 2022). In support of this, there is evidence indicating that T2D patients have hippocampal atrophy (Gold et al., 2007; Hayashi et al., 2011; Moran et al., 2013), or reduced bilateral hippocampal volumes (Li et al, 2021; Milne et al., 2018). However, to date, there is a research-gap on how acute stress affects declarative memory in older adults with T2D, since no previous studies have been carried out.

Therefore, the aims of this study were to compare, firstly, the psychobiological (i.e., affect, sAA and salivary cortisol) response to an acute psychosocial stress in older people with T2D with a group of age and sex matched healthy participants, and secondly, the acute stress impact on hippocampus-dependent memory retrieval performance. We hypothesized, based on previous studies (Buckert et al., 2022; Faulenbach et al., 2013; Vallejo et al., 2021) that the psychobiological response will be higher in the stress condition and, specifically, T2D participants will display stronger, at least, cortisol response (Bucker et al., 2022) than healthy participants. In addition, according to previous findings in healthy older people (Pulopulos et al., 2013), we expect no effects of acute stressor on memory retrieval performance.

Material and Methods

Participants

The sample was composed of 100 participants (50 T2D and 50 non-T2D) from 52 to 80 years old. Both groups were randomly assigned to two conditions in a counter-balanced way: T2D (24 to the stress condition and 26 to the control condition), and non-T2D or healthy participants (24 to the stress condition and 26 to the control condition).

T2D participants were recruited from medical clinics and hospitals and referred by their doctors. They were medically treated and controlled during the period of the research. Non-T2D participants proceeded from a study program at the University of Valencia for people over 55 years. Exclusion criteria were as follows: abuse of alcohol or other drugs of abuse, smoking more than 10 cigarettes per day, presence of severe cardiovascular disorder, neurological or psychiatric disorder, visual or hearing impairments, the presence of a stressful life event in the past year, having been under general anaesthesia in the past year, and consuming drugs related to emotional or cognitive functions or psychotropic substances. Additionally, participants who used beta-blockers, glucocorticoids, asthma medication, antidepressants or thyroid therapies were excluded from the study. The absence of cognitive impairment was considered after administration of the Spanish version of the Mini-Mental Status Examination (MEC; Lobo et al., 1999), excluding those individuals with a score of less than 28.

Memory assessment

Picture recall

Participants were shown 30 colour pictures (10 negative, 10 positive and 10 neutral) from the International Affective Picture System (IAPS; Lang et al., 2005). Pictures were presented individually on a computer screen, for 5 seconds when the screen appeared black, participants were to rate the emotional valence

(from 1 = very negative to 9 = very positive) and arousal (from 1 = low arousal to 9 = high arousal) of the images using the Self-Assessment Manikin (SAM; Lang, 1980).

The following day, the task consisted of recalling the largest number of images seen the previous day. Participants had 10 min to describe a short description of the pictures in written form. Two independent judges (LV and MZ-F) delimited which picture corresponded to each description. Discrepancies between judges were resolved by consensus.

Rey Auditory Verbal Learning Test (RAVLT)

Declarative memory was assessed with the Rey Auditory Verbal Learning test (RAVLT; Spanish version of Miranda and Valencia, 1997). The RAVLT consists of reading a 15 wordlist during 5 consecutive trials, followed by a presentation of an interference list. After that, two recall trials are presented. The first is done after the recall of the interference list (immediate recall), and the second after a delay of 20 min (20m-delayed recall). One day after, participants performed a delayed free recall task (1day-delayed recall).

Rivermead stories subtest

The Story Recall subtest from the Spanish version of the Rivermead Behavioral Memory Test (Wilson et al., 1985) consist of reading aloud two brief stories by the experimenter. Then, the subjects had to recall as many “ideas” as possible just immediately after their presentation (immediately recall), and after a 20-min delay (delayed recall). The total of the recalled “ideas” from the stories was calculated. Three outcomes were used for the analysis: i) Immediate Recall: sum of “ideas” referred from the two stories; ii) 20m-delayed recall: sum of “ideas” referred from the two stories after 20 min of delay; iii) 1-day delayed recall: sum of “ideas” referred from the two stories after one day.

Psychological Assessment

Self-Efficacy

Participants' self-efficacy, understood as beliefs about one's capacity to organize and carry out actions (Bandura, 1997), was evaluated with three items (van der Meij et al., 2010; Villada et al., 2017). These items were formulated to measure capacity ("How capable are you of successfully performing the task?"), self-confidence ("How confident are you that you will successfully perform the task?"), and importance of successfully performing the task ("How important is it to you to perform this task successfully?"). Participants had to respond on a Likert scale ranging from "1 = not at all" to "100 = totally."

Situational Appraisal

Participants' situational appraisal was measured by several items (Gonzalez-Bono et al., 2002), focusing on the following aspects: frustration, motivation, amount of perceived stress, difficulty of the task, assessment of their own performance, expected outcome of the evaluation, and perception of the effort required (e.g., How much effort did the task require?). Each item was rated on a 5-point Likert scale (not at all = 1 to extremely = 5).

Mood

We employed the Spanish version (Sandín et al., 1999) of the Positive and Negative Affect Scale (PANAS) (Watson et al., 1988). This 20-item questionnaire assesses mood in two dimensions: positive affect (PA: interested, excited, strong, enthusiastic, etc.) and negative affect (NA: distressed, upset, guilty, scared, etc.), with 10 items measuring each state. The participants were asked to complete the questionnaire based on how they felt at that moment. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). In the current study, Cronbach alpha coefficients were 0.91 and 0.87 for PA and NA, respectively.

Procedure

This study consisted of two individual sessions: acquisition and retrieval. In the first session (acquisition session), participants were asked to assist to the Laboratory of Social Cognitive Neuroscience at the University of Valencia between 10:00 and 12:00 h. There, firstly, the experimenter verified that the participants had followed the instructions before both sessions: drink only water and not eat, smoke, take any stimulants (e.g., coffee, cola, tea, chocolate), or brush their teeth at least 2 h prior to the sessions. In addition, volunteers had to sleep as long as usual, avoid heavy physical activity from the evening before sessions and not consume alcohol since the night before sessions. Secondly, after a period of habituation to the laboratory, participants were administered the MEC, the picture encoding task, the RAVLT, and the Rivermead subtest. They were not informed that the next day they would have to attempt to recall the pictures, the RAVLT word list, and the Rivermead material. Two saliva samples (before and after the memory assessment) were obtained from all participants to measure cortisol level).

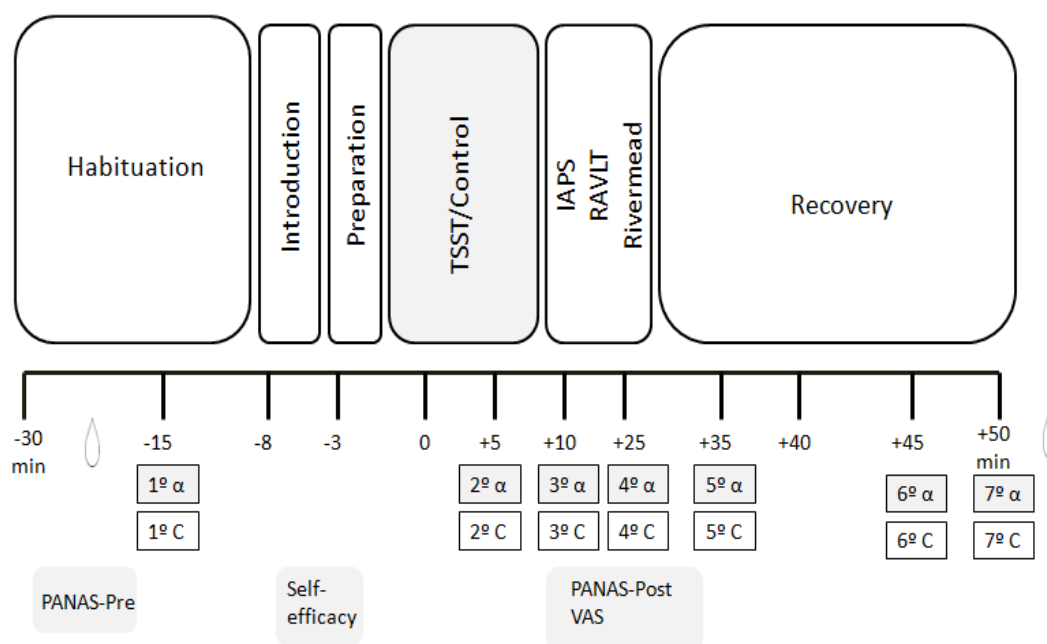
The second session (retrieval session) was held between 16:00 and 18:00 h in the same laboratory. To produce stress, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) was employed. Participants in the stress group were exposed to 5 min of free speech (simulated job interview) and 5 min of an arithmetic task. The control task consisted of 5 min of free speech about a non-emotional experience followed by the arithmetic task (counting by 5 aloud during 5 min). The control task has been used in previous studies (Almela et al., 2011; Hidalgo et al., 2012; Pulpulos et al., 2013). Before the TSST or control task, participants completed several items to assess the PANAS (pre-task PANAS) and the perceived self-efficacy to obtain the baseline measure for mood. Immediately after the TSST/Control task, participants filled Situational Appraisal (some questions about the task and the PANAS (post-task PANAS). In addition, after completing the stress/control task, participants performed the free recall task of images viewed the previous day. After this, the free recall task of the RAVLT word list and the Rivermead stories were performed.

During the retrieval session, seven saliva samples were collected during each session. Prior to the stress/control task, the first saliva sample was collected (habituation: -15 min pre-stress). The rest of the saliva samples were taken during the stress/control task (+5 min), after the TSST/control task (+10 min post-stress), three at 10 min periods after the tasks (+25, +35, and +45 min), and finally at +50 min post-stress.

To measure glucose concentrations, two capillary blood samples were taken at habituation (-20 min) and in the recovery period (+55 min). The same time schedule and phase durations for sample collection were followed by both groups and conditions. The questionnaires administered only differed on the task (stress vs. control) (see Figure IV.1).

All subjects received verbal and written information about the study and signed an informed consent form. The study was carried out in accordance with the Declaration of Helsinki, and the Ethics Research Committee of the University approved the protocol.

Figure IV.1. Timeline of the retrieval session (second day) for the TSST and control conditions. Salivary cortisol samples = 1^o C, 2^o C, 3^o C, 4^o C, 5^o C, 6^o C, 7^o C. Salivary alpha-amylase samples = 1^o α, 2^o α, 3^o α, 4^o α, 5^o α, 6^o α, 7^o α; Time of collection of saliva samples (-15, +5, +10, +25, +35, +45, +50); TSST= Trier Social Stress Test; PANAS= Positive and Negative affect; VAS= Valence and arousal scale. IAPS= International Affective Picture System; RAVLT= Rey Auditory Verbal Learning Test; Rivermead= Rivermead stories subtest.



Biochemical Analyses

Salivary samples were collected using salivettes (Sarstedt, Nümbrecht, Germany). Participants were instructed to keep the cotton swab in their mouths for 2 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva. The samples were kept in the refrigerator until they were centrifuged at 3,000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was stored at -80 C until the analyses. For each subject, all cortisol and sAA samples were analyzed in the same trial.

Cortisol levels were analyzed using the commercial enzymeimmunoassay kit from Salimetrics (Newmarket, United Kingdom). Assay sensitivity was <0.007 µg/dL, and the intra- and inter-assay coefficients of variation were all below 10%. Cortisol levels were expressed in nmol/L.

The sAA concentration was measured by using an enzyme kinetic method with the commercial salivary sAA assay kit from Salimetrics (United States). Assay sensitivity was 0.4 U/mL. Inter- and intra-assay variation coefficients were all below 10%. sAA concentrations were expressed in U/mL.

To measure glucose concentration two capillary blood samples were taken at habituation (-20 min) and recovery period (+55) using a glucose monitoring system (Onetouch ultraeasy life Scan Europe 6300 Zug, Switzerland, AW 0639870).

Statistical analysis and data management

Data were checked for normal distribution and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests before the statistical procedures were applied. Since neither the sAA nor the cortisol data had a normal distribution, they were log transformed.

To investigate Condition (stress vs. control) and Group (T2D vs. healthy) differences in sociodemographic variables, Student's *t*-test and X^2 were used depending on the nature of the variable.

Multivariate analyses of variance (MANOVA) with each self-efficacy (i.e., capacity, self-efficacy and motivation), and situational appraisal (i.e. effort

required, frustration, own performance, stress perceived, difficulty, importance of doing the task, and expected outcome) item as dependent variables, and Condition and Group as independent variables.

Analyses of variance (ANOVA) for repeated measures were performed to investigate Condition and Group differences in glucose levels, affect, sAA and cortisol levels with Condition and Group as between-subject factors, and Time (glucose and affect: pre vs. post session; sAA and cortisol: -15, +5, +10, +25, +35, +45, and +50 min) as within-subject factor.

ANOVAs for repeated measures were also performed to investigate Condition and Group differences in learning and memory performance (acquisition session) on RAVLT and Rivermead tasks with Condition and Group as between-subject factors, and Time (immediate recall and (20m-delayed recall) as within-subject factor.

To explore Condition and Group differences on delayed recall (retrieval session), an ANOVA for repeated measures was performed for pictures, with Condition and Group as between-subject factors, and Valence (positive, negative and neutral) as within-subject factor. In addition, two-way ANOVAs were used for words and paragraphs (dependent variable: 1-day delayed recall of RALVT and Rivermead tests).

Finally, to assess whether sAA and cortisol responses to stress were related to words, paragraphs and pictures delayed recall, bivariate Pearson's correlations were conducted between the delayed recall for words, paragraphs, positive, negative and neutral pictures and *sAA reactivity* (maximum sAA levels after the onset of TSST minus baseline period) and *Cortisol reactivity* (maximum cortisol levels after the onset of TSST minus baseline period).

Greenhouse–Geisser was used when the requirement of sphericity was violated in the ANOVA for repeated measures. *Post hoc* planned comparisons were performed using Bonferroni adjustments for the *p*-values. Analyses were carried out using SPSS 28.0. All *p* values reported are two-tailed. The level of significance was fixed as <.05. The values in the figures represent raw values, for easy interpretation.

Results

Preliminary analyses

Sociodemographic variables No condition differences were found on age ($p = 0.266$), sex ($p = 0.701$), education level ($p = 0.570$), SES ($p = 0.968$), and BMI ($p = 0.743$). No group differences were found on age ($p = 0.419$), sex ($p > 0.999$), SES ($p = 0.134$) and BMI ($p = 0.458$). However, group differences were found in educational level ($p < 0.001$). Thus, among T2D participants, 17 reached Basic studies, 14 High School and 15 College or higher and 3 had no studies, while among healthy participants, 18 reached High School, 25 College or higher and 7 had no studies.

Glucose levels There was a main effect of Group ($F(1, 95) = 38.299, p < 0.001$), and Time ($F(1, 95) = 4.686, p = 0.033$). Thus, T2D group had higher glucose levels than healthy group, and all participants had higher glucose levels before than after the retrieval session. In addition, the interaction between these two factors was significant ($F(1, 95) = 6.168, p = 0.015$). *Post hoc* analyses revealed that T2D participants had higher glucose levels in both pre and post session in comparison to healthy participants (both $p < 0.001$), and they had higher glucose levels pre than post session ($p = 0.002$). However, healthy participants had similar glucose levels between times ($p = 0.821$).

Self-efficacy Condition effects were found in capacity ($F(1, 95) = 16.366, p < 0.001$), self-confidence ($F(1, 95) = 8.513, p = 0.004$), and motivation ($F(1, 95) = 4.611, p = 0.034$). Thus, participants in the stress condition reported less capacity, confidence and motivation to successfully perform the task, in comparison to participants in the control condition.

Regardless of condition, diabetic participants reported less confidence than healthy participants ($F(1, 95) = 4.390, p = 0.039$), but not capacity ($F(1, 95) = 2.745, p = 0.101$), nor motivation ($F(1, 95) = 0.387, p = 0.535$). The interaction

between Condition and Group was not significant in any dimension (all $p > 0.374$).

Situational appraisal. The stress task was perceived as more frustrating ($F(1, 92) = 62.217, p < 0.001$), stressful ($F(1, 92) = 53.674, p < 0.001$), difficult ($F(1, 92) = 136.684, p < 0.001$), and requiring more effort ($F(1, 92) = 58.248, p < 0.001$) than the control task. However, participants in the control condition perceived a better performance ($F(1, 92) = 86.726, p < 0.001$), and result ($F(1, 92) = 82.947, p < 0.001$) on the control task in comparison to those of stress condition. The importance of doing well the task was similar between conditions ($F(1, 92) = 1.538, p = 0.218$). In addition, both T2D ($p = 0.003$) and healthy ($p < 0.001$) participants perceived the TSST more stressful than control task. In the stress condition, T2D participants perceived less stressful the TSST than healthy participants ($p = 0.007$). No group differences were found in the perception of stress of the control task ($p = 0.195$).

Psychobiological stress response

Affect

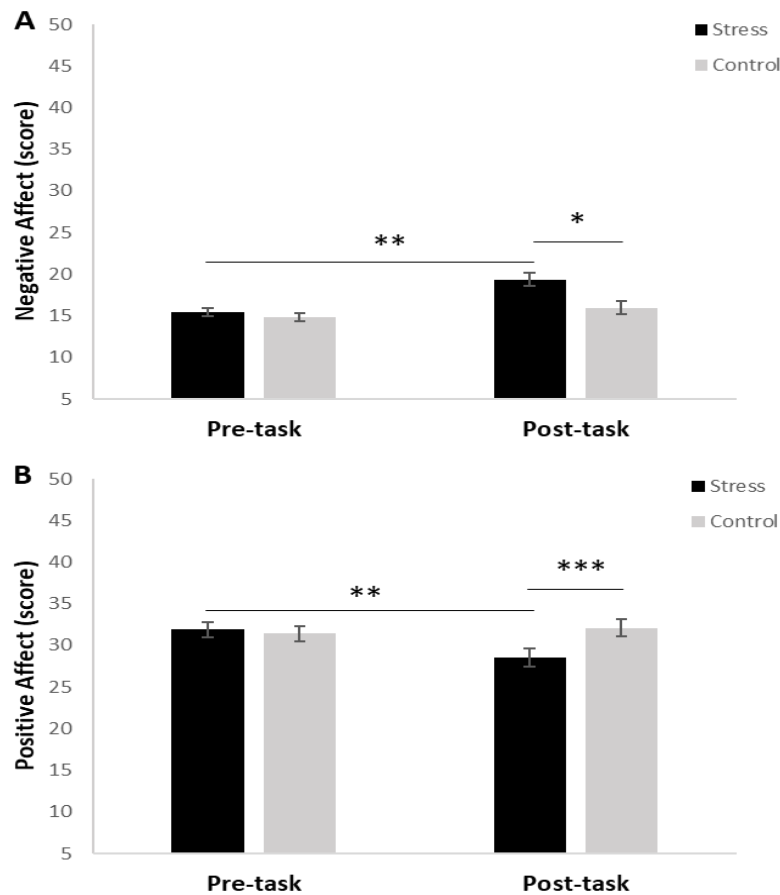
Negative affect The negative affect scores were higher in the stress than in the control condition ($F(1, 94) = 6.394, p = 0.013$), in T2D in comparison to healthy participants ($F(1, 94) = 10.152, p = 0.002$), and post-task than pre-task ($F(1, 94) = 27.911, p < 0.001$). The Time x Condition ($F(1, 94) = 8.060, p = 0.006$), and Time x Group ($F(1, 94) = 5.790, p = 0.018$) interactions were significant. *Post hoc* analyses showed that, after TSST negative affect was higher than after control task ($p = 0.003$). No condition differences were found before tasks ($p = 0.366$). In addition, negative affect scores were higher, significantly, after TSST than before ($p < 0.001$), and marginally after the control task than before ($p = 0.081$).

Finally, the T2D participants scored higher before TSST or control tasks ($p < 0.001$) than healthy participants, while no group differences were found after

tasks ($p = 0.212$). Both T2D and healthy participants presented higher negative affect scores after than before tasks (both $p > 0.047$) (Figure IV.2A).

Positive affect Regardless condition and group factors, participants had higher positive affect before than after tasks ($F(1, 94) = 5.927, p = 0.017$). The Time x Condition interaction was significant ($F(1, 94) = 13.945, p < 0.001$). Thus, participants in the stress condition had lower positive affect than participants in the control condition only after ($p = 0.020$), and not before ($p = 0.703$) task, and after the TSST than before ($p < 0.001$). The Condition ($F(1, 94) = 1.372, p = 0.245$) nor Group ($F(1, 94) = 0.849, p = 0.359$) factors, neither the rest of interactions were significant (all $p > 0.528$) (Figure IV.2B).

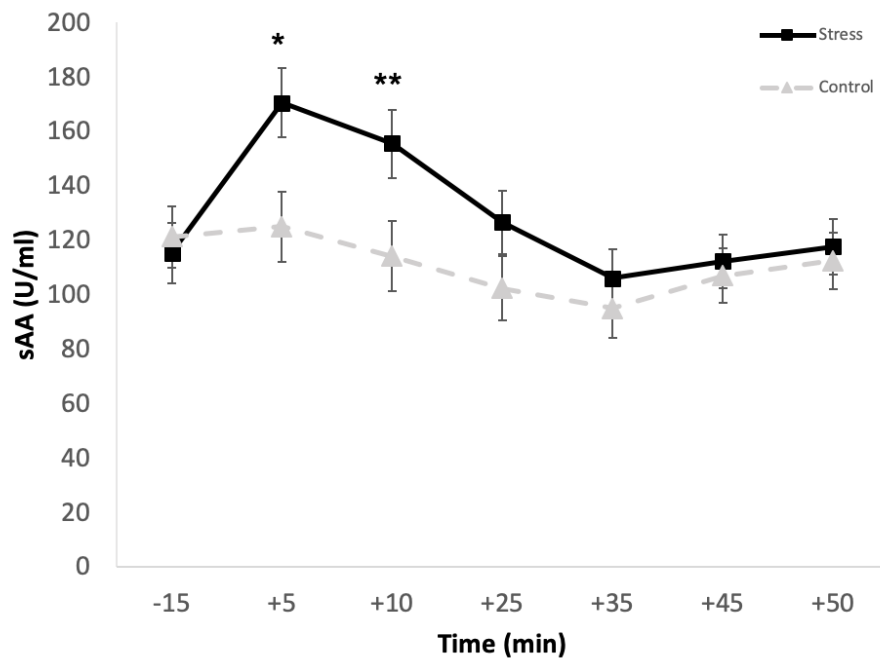
Figure IV.2. Negative (A) and Positive (B) affect pre and post stress or control tasks (* $p = 0.003$; ** $p < 0.001$); *** $p = 0.020$)



Salivary alpha-amylase response

Results revealed that the Group ($F(1, 89) = 1.450, p = 0.232$) and Condition ($F(1, 89) = 1.028, p = 0.313$) factors were not significant, nor were the Group x Condition ($F(1, 89) = 0.409, p = 0.524$), Time x Group ($F(4.014, 357.261) = 0.951, p = 0.435$), and Time x Group x Condition ($F(4.014, 357.261) = 1.415, p = 0.228$) interactions. However, the Time factor ($F(4.014, 357.261) = 18.120, p < 0.001$) and the Time x Condition interaction ($F(4.014, 357.261) = 7.717, p < 0.001$) were significant. No baseline differences were found between conditions ($p = 0.384$), however participants in the stress condition showed higher cortisol levels than participants in the control condition during the task (+5 min, $p = 0.035$), and immediately after the task (+10 min, $p = 0.028$) samples. There were no condition differences in the rest of the samples (all $p > 0.128$). In the stress condition, sAA levels increased during the TSST in comparison to baseline (-15 vs +5: $p < 0.001$), remaining similar after 5 min (+5 vs +10: $p > 0.999$). Then, sAA levels started to decrease 15 min (+10 vs +25: $p < 0.001$), and 25 min (+25 vs +35: $p < 0.001$) after the end of the TSST and remaining stable 35 min (+35 vs +45: $p > 0.999$) and 40 min (+45 vs +50: $p > 0.999$) after the onset of the TSST. Participants recovered baseline levels (+50 vs -15: $p > 0.999$). In the control condition, no significant sAA levels changes (increase or decrease) compared to baseline were found (all $p > 0.999$), except 35 min after the onset of the TSST, sAA levels were lower than baseline (-15 vs +35: $p = 0.007$) (Figure IV.3).

Figure IV.3. Salivary alpha-amylase levels in the stress and control conditions for the retrieval session (* $p = 0.035$; ** $p = 0.028$).

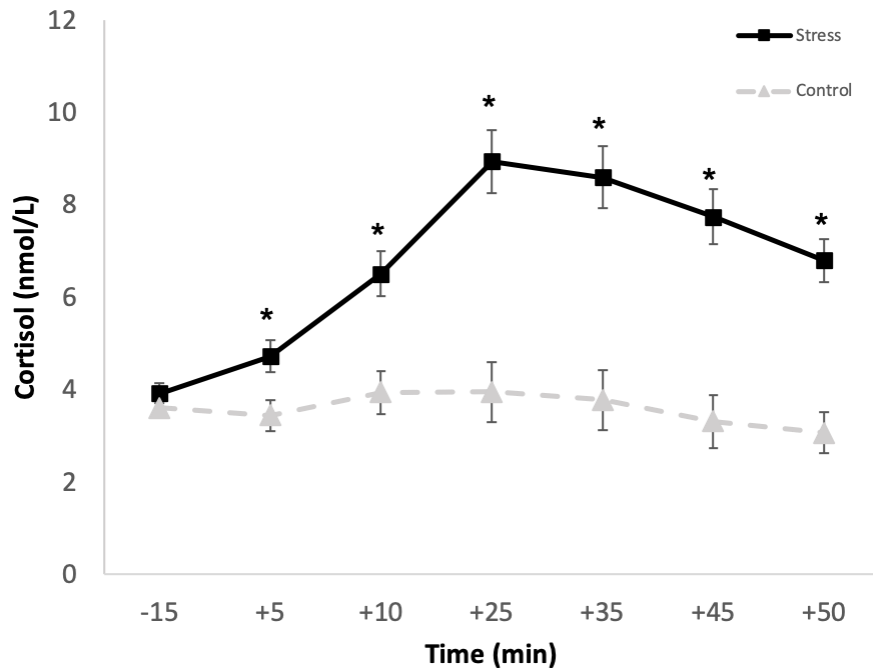


Salivary cortisol response

Results showed that Time ($F(2.271, 217.985) = 17.931, p < 0.001$) and Condition ($F(1, 96) = 18.812, p < 0.001$) factors, and Time x Condition interaction ($F(2.271, 217.985) = 19.132, p < 0.001$) were significant. Thus, participants in the stress condition secreted more cortisol than those of the control condition. Except in the baseline sample ($p = 0.348$), participants in the stress condition had higher cortisol levels than participants in the control condition in all samples (all $p > 0.022$). In the stress condition, baseline cortisol levels were similar than those during the TSST (-15 vs. +5: $p = 0.238$), but, immediately after the TSST (+5 vs +10: $p < 0.001$) they increased reaching the peak 15 min (+10 vs +25: $p < 0.001$), and remaining similar 25 min (+25 vs +35: $p > 0.999$) and 35 min (+35 vs +45: $p > 0.999$) after the end of TSST. At the end of the retrieval session, participants did not recover baseline levels, given that they had higher cortisol levels (+50 vs +15: $p < 0.001$). In the control condition, no significant cortisol levels changes (increase or decrease) compared to baseline were found (all $p > 0.999$) (Figure

IV.4). The Group ($F(1, 96) = 0.292, p = 0.590$) factor nor their interactions were significant (all $p > 0.092$).

Figure IV.4. Salivary cortisol levels in the stress and control conditions for the retrieval session (*all $p > 0.022$).



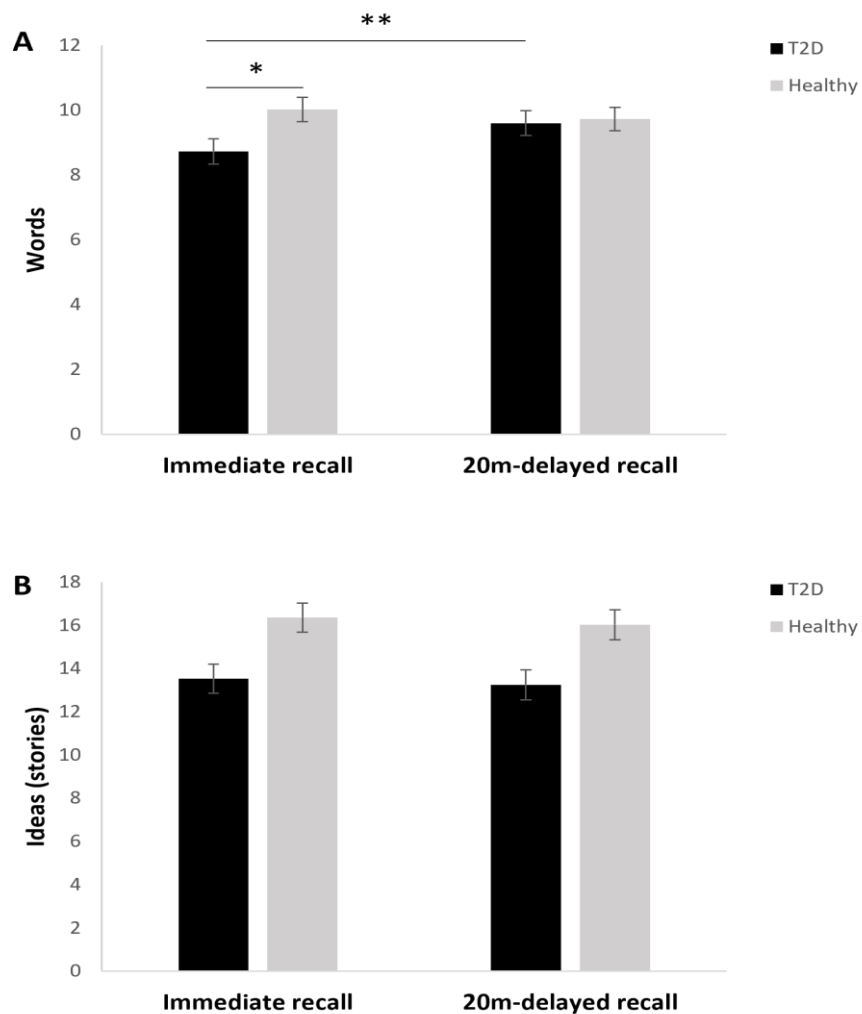
Declarative memory performance

Acquisition session

RAVLT Participant in both conditions ($F(1, 92) = 0.086, p = 0.771$), groups ($F(1, 92) = 1.955, p = 0.165$) and times ($F(1, 92) = 3.020, p = 0.086$) recalled a similar number of words. However, the Time x Group interaction ($F(1, 92) = 12.341, p < 0.001$) was significant. Thus, T2D participants recalled less words than healthy participants immediately after the words acquisition ($p = 0.019$), but no group differences were found after 20 min delayed period ($p = 0.806$). In addition, diabetic participants recalled less words immediately than 20 min after the exposition to words ($p < 0.001$), while healthy participants did not differ in their performance in both trials ($p = 0.203$). No other interactions were significant (all $p > 0.512$) (Figure IV.5A).

Rivermead stories Overall, the performance of the diabetic participants was lower than those of the healthy group ($F(1, 96) = 9.127, p = 0.003$). The Condition ($F(1, 96) = 1.193, p = 0.277$) and Time ($F(1, 96) = 1.303, p = 0.256$) factors were not significant. No significant differences were found in all interactions (all $p > 0.256$) (Figure IV.5B).

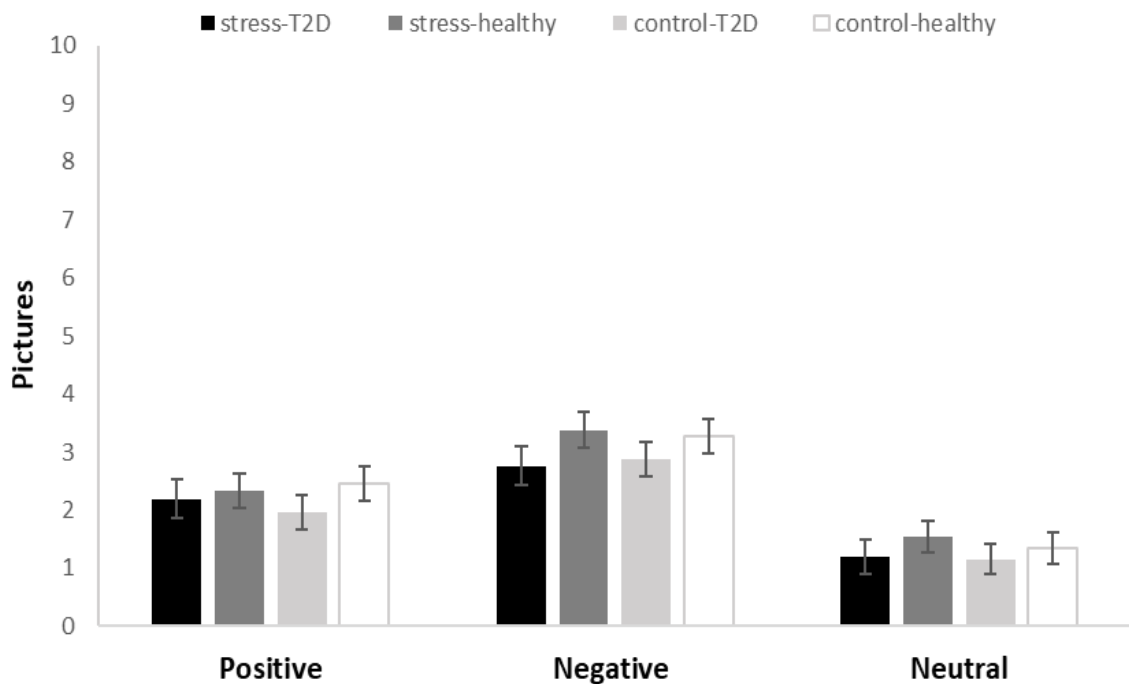
Figure IV.5. Declarative memory performance in the acquisition session. Immediate and 20 min delayed recall of words (A) and ideas of stories (B) (* $p = 0.019$; ** $p < 0.001$).



Retrieval session

Pictures recall Results revealed a main effect of Valence ($F(2, 186) = 60.831, p < 0.001$), being the negative pictures more recalled than positive ($p < 0.001$), and neutral pictures ($p < 0.001$), and positive pictures were more recalled than neutral ones ($p < 0.001$). In contrast, Condition ($F(1, 93) = 0.051, p = 0.821$) or Group ($F(1, 93) = 2.456, p = 0.121$) factors were not significant, neither the interactions between main factors (all $p > 0.608$) (Figure IV.6).

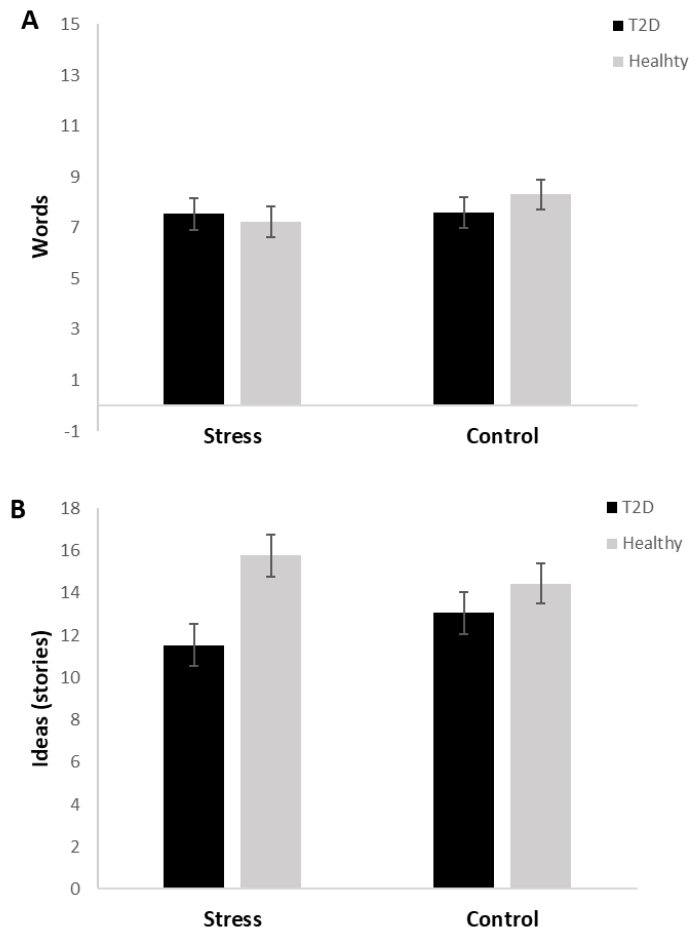
Figure IV.6. Number of positive, negative and neutral pictures recalled in the retrieval session for T2D and healthy participants in the stress and control conditions.



RAVLT There were no significant differences between stress and control conditions ($F(1, 92) = 0.818, p = 0.368$) or T2D and healthy participants ($F(1, 92) = 0.125, p = 0.724$). The Condition $\times$ Group interaction was also not significant ($F(1, 92) = 0.709, p = 0.402$) (Figure IV.7A).

Rivermead stories T2D participants recalled stories worse than healthy participants ($F(1, 93) = 8.037, p = 0.006$). However, stress did not affect the performance of this subtest ($F(1, 93) = 0.009, p = 0.923$). The interaction between Condition and Group was not significant ($F(1, 93) = 2.073, p = 0.153$) (Figure IV.7B).

Figure IV.7. Number of words and ideas of stories recalled in the retrieval session for T2D and healthy participants in the stress and control conditions.



Relationship between sAA and cortisol response to stress and declarative memory performance

In T2D participants of the stress condition, no relationships between sAA and cortisol stress response and free recall of positive (sAA: $r = -0.057$, $p = 0.805$; cortisol: $r = -0.208$, $p = 0.366$), negative (sAA: $r = 0.136$, $p = 0.556$; cortisol: $r = -0.065$, $p = 0.781$) or neutral (sAA: $r = -0.125$, $p = 0.588$; cortisol: $r = -0.054$, $p = 0.817$) pictures, and RAVLT (sAA: $r = 0.049$, $p = 0.828$; cortisol: $r = -0.136$, $p = 0.547$) and Rivermead stories (sAA: $r = -0.013$, $p = 0.953$; cortisol: $r = -0.091$, $p = 0.681$) were found.

Similarly, in healthy participants of the stress condition, sAA and cortisol stress response were not related to free recall of positive (sAA: $r = 0.059$, $p = 0.786$; cortisol: $r = 0.228$, $p = 0.285$), negative (sAA: $r = 0.014$, $p = 0.948$; cortisol: $r = 0.134$, $p = 0.532$) or neutral (sAA: $r = -0.049$, $p = 0.819$; cortisol: $r = 0.362$, $p = 0.083$) pictures, and RAVLT (sAA: $r = -0.354$, $p = 0.090$) and Rivermead stories (sAA: $r = -0.075$, $p = 0.726$; cortisol: $r = 0.140$, $p = 0.514$). However, cortisol response was negatively related to RAVLT performance marginally ($r = -0.397$, $p = 0.055$).

Discussion

The aims of the current study were to study the psychobiological response and the impact of acute stress on declarative memory performance in older people with T2D in comparison to age and sex healthy counterparts. The main results were that the T2D participants reported a similar psychobiological response than healthy participants. In addition, stress did not affect the memory retrieval for pictures, words or stories in both T2D and healthy groups. Regardless of condition, T2D participants recalled more ideas of paragraphs than healthy ones. Finally, only in healthy participants, cortisol reactivity to stressor was marginally negatively related to word-free recall.

As we hypothesized, the stressor used (i.e., TSST) elicited a robust psychobiological response in all participants, T2D and healthy people. Participants perceived the stress task as more frustrating, stressful and difficult, and requiring more effort than the control task. In addition, participants in the stress condition perceived a lower performance and result than those in the control condition, and all participants showed a similar motivation to perform the task. Although T2D participants perceived the TSST less stressful than healthy people, the stress task produced changes at psychological level, since after it, all participants reported lower positive affect and higher negative affect. This result is in line with previous studies included only healthy older adults (Hidalgo et al., 2020) and T2D older people (Vallejo et al., 2021). Moreover, no baseline difference was found in positive affect, but T2D had higher negative affect before the onset of task.

A “typical” sAA response to TSST was found in T2D in comparison to healthy individuals. This result is in line with previous studies in older people (for a review on this topic see: Pulpulos et al., 2018), and extend the findings found by Vallejo et al. (2021) who only included T2D patients. However, our result is in contrast to the attenuated sAA response in older people reported by Strahler et al. (2010). At this point, it is important to note that, although the two studies used the TSST, Strahler et al. (2010) used a shorter preparation phase (3 min), while the current study used a longer preparation phase (5 min), so the duration of the anticipatory phase was different. In addition, the sample included in the study carried out by Strahler et al. (2010) was younger (59-61 years old) than our sample (52-80 years old). Maybe, these differences could help us to understand this mixed result.

All participants, diabetic and healthy, in the two conditions, had similar baseline cortisol levels, however, since five min after the onset of the task to the end of the

session, participants in the stress condition showed higher salivary cortisol levels than participants in the control condition, demonstrating that the TSST provoked a stronger cortisol response than control task. This finding is in line with previous studies that exposed only T2D (Faulenbach et al., 2013; Vallejo et al., 2021) or T2D and healthy (Buckert et al., 2022) participants to the TSST. However, it is in contrast to Steptoe et al. (2014) who found a blunted cortisol response to a mild mental stressor. Specifically, T2D had higher cortisol concentrations than healthy participants across the entire session, but they did not show a cortisol response to the stressor, in contrast to the weak cortisol response reported to the healthy group (Steptoe et al., 2014). As Buckert et al. (2022) discussed, the different stressors applied with different ability to activate the HPA axis could explain the discrepant results, having more potential to activate the HPA axis the TSST than the mental stressor (Allen et al., 2017; Dickerson and Kemeny, 2004).

In contrast to the stronger cortisol response found in T2D patients in comparison to healthy group by Buckert et al. (2022), we failed to find an effect of group in the cortisol response to TSST, that is, the cortisol response was similar between T2D and healthy participants, not confirming our hypothesis. It should be noted that the biological sample used to measured cortisol concentrations was different across the studies. While in the Buckert et al. (2022), the cortisol response was measured with blood samples, in the current study, we used saliva samples. It is possible that, on the one hand, the fact that there is a higher concentration of cortisol in blood than in saliva and that, on the other hand, the puncture to obtain the blood sample is more invasive than the collection of the saliva sample and, therefore, is more stressful, cortisol levels may be biased. Further studies are needed to test these possible explanations.

Together, this is the first study reporting a similar psychobiological response in T2D older people that healthy older people of both sexes comparing directly both groups. Maybe, this similar stress response could be due to all participants had similar motivation to perform the task, and/or because the T2D participant were medically treated.

As expected, regardless of the group, no significant stress effect on memory retrieval was observed for pictures, words or stories. Our results agree with those observed in healthy older people in declarative memory (Pulopulos et al., 2013; Hidalgo et al., 2014, 2015), and extent those previously found in T2D patients without comparison to healthy control group in working memory (Vallejo et al., 2021). The lack of stress effects on declarative memory performance is in line with the hypothesis that memory in older people might be less sensitive to the acute effects of psychosocial

stress-induced cortisol increases than young people, as we previously suggested in a revision (Hidalgo et al., 2019). This age group suffer important changes in SNS (i.e., as a reduction in the locus coeruleus integrity and a decline in noradrenergic modulation) (Arnsten and Goldman-Rakic, 1985a, 1985b), and a dysregulation of the HPA axis functioning (i.e., decrease in the number and sensitivity of cortisol receptors) (Mizoguchi et al., 2009), and functional alterations in both amygdala and hippocampus (Mather, 2006; Murty et al., 2009; St. Jaques et al., 2009).

In addition, we found that diabetic participants recalled a smaller number of ideas about stories in both, acquisition and retrieval session, and recalled less words only immediately after the words acquisition than healthy participants. However, no group differences were found in 20-min and 1-day delayed free recall of words and free recall of pictures. These results could be explained by the fact that among diabetic patients is common an atrophy of the hippocampus (Gold et al., 2007; Hayashi et al., 2011; Moran et al., 2013) since in this brain area are located large amount of insulin receptors (Pomytkin et al., 2018). Considering that declarative memory is a cognitive domain dependent on hippocampal functioning and, according to Lezak et al. (2004) a paragraph recall test is considered to be a clearest measure of declarative memory in comparison to words and pictures test, is conceivable that diabetic people had lower performance in immediate and 20-min and 1-day delayed free recall on the stories test.

Finally, only in healthy participants, cortisol reactivity to stressor was marginally negatively related to word-free recall. Thus, healthy participants who responded to the stress task with higher cortisol concentrations recalled, marginally, less words. This finding is in line with previous studies carried out with young people where reported a negative association between cortisol response to stress and memory retrieval (Buchanan and Tranel, 2008; Buchanan et al., 2006; Domes et al., 2004; Hidalgo et al., 2015; Smeets, 2011; Smeets et al., 2008). However, our result is in contrast to those reported by Pulpulos et al. (2013) who failed to find an association between stress-induced cortisol response and memory retrieval for pictures, words and stories in older people. One explanation of this controversial results could be that the cortisol reactivity was calculated differently. In the current study, we calculated the cortisol reactivity subtracting the basal levels from the maximum levels after the onset of the stressor, while Pulpulos et al. (2013) calculated this cortisol index with the area under the curve with respect to increase (AUCi), according to the trapezoid formula specified in Pruessner et al. (2003). Future research may help to clarify this issue.

The current study has some limitations that led us to interpret our results with caution. Firstly, the number of the participants in each group was limited. Secondly, and in line with the above, despite the evidence suggesting the sex differences on both stress-related cortisol response (see review Pulpulos et al., 2018), and in stress-related effects on memory retrieval (see review Hidalgo et al., 2019), we did not study sex differences remaining unknown whether there are age differences in the psychobiological response to stress, as well as its effects on declarative memory in T2D patients. Hence, future studies should include more participants in order to investigate possible sex differences. Thirdly, although the three memory tasks used assessed declarative memory, the order of the administration was not counterbalanced. Further studies should counterbalance the order of the memory tests in order to confirm this lack of stress effects on memory retrieval.

Conclusions

In sum, our study provides empirical evidence reporting that T2D patients show a similar psychobiological response to an acute psychosocial stressor and extend the previous idea suggesting older people are less sensitive to stress effects on memory retrieval in older T2D patients.

Chapter 5

Study 3: Diurnal cortisol cycle and cognitive performance in Type 2 Diabetes

Introduction

Type two diabetes (T2D) has risen to become one of the most important public health problems of the 21st century worldwide (Hu et al., 2017). T2D is characterised by abnormally disturbed glucose metabolism, with lower glucose absorption, translating into micro and macrovascular complications and chronic hyperglycaemia (Biessels et al., 2017).

Diabetes is considered a chronic metabolic stressor with several important consequences for those who suffer it. Hyperglycemia leads to a diminished feedback regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, and there is evidence about HPA axis hyperactivation with increased cortisol (C) release in T2D (Sharma et al., 2020). In the last decades, the association of T2D with cognitive deficits (Antal et al., 2022; Damanik & Yunik, 2021) and with mild cognitive impairment (MCI) and Alzheimer disease (AD) (Kim, 2019) has been highlighted although the underlying mechanisms are not well known (Marissal-Arvy & Moisan, 2022). Since T2D has been related to stress, the HPA functioning and its dysregulation has been cited as a potential mechanism of the cognitive dysfunctions associated with this disease (Johar et al., 2016; Joseph & Golden, 2017). In fact, a dysregulation of the HPA axis has been related to cognitive problems in healthy people (Gardner et al., 2019; Lupien et al., 2005; Almela et al., 2012; Hidalgo, et al., 2016). Thus, the study of the HPA axis functioning in T2D patients has gained increasing interest (Seal & Turner, 2021; Sunena & Mishra, 2022).

Normally, the cortisol circadian rhythm shows a pattern characterized by cortisol rising post-awakening, peaking at 30–45 min, known as C awakening response (CAR) and gradually declining over the following hours to reach the lower levels at the end of day. The difference in C from morning to evening permits to calculate the Diurnal Cortisol Slope (DCS) (Adam et al., 2017). Overall, a disturbed functioning of the HPA axis in T2D people associated with greater severity of clinical deficits has been stated (Chiodini et al., 2007). Specifically, a flattened CAR has been reported in T2D when compared with non T2D individuals (Bruehl et al., 2009; Panagiotou et al., 2021; Lederbogen et al., 2011).

although not always (Hackett et al., 2014). Similarly, a blunted DCS has been found (Hackett et al., 2014; Lederbogen et al., 2011) whereas in other studies differences with healthy controls were not described (Bruehl et al., 2009;). Therefore, the results are heterogeneous being important to establish more clearly how T2D is related to a dysregulation of the HPA.

The dysfunction of the HPA axis would affect the hippocampus, having evidence of atrophy in this cerebral structure among older T2D patients (Bruehl et al., 2009; Den Heijer et al., 2003; Ho et al., 2013; Du et al., 2021) as well as in the prefrontal cortex (PFC) (Stomby et al., 2016; Lebedeva et al., 2018). T2D has been associated with smaller total and regional brain volume and greater atrophy over time (Zhang et al., 2022). In addition to brain changes, further decreased cognitive performance has been reported (Bruehl et al., 2009; Milne et al., 2017). Yau et al. (2014) reported declarative memory (DM) impairments associated hippocampal volume reduction in middle-age and elderly patients with T2D. There are several meta-analyses that report evidence of small to moderate effect sizes in domains such as verbal and visual memory, processing speed and executive function (EF) (Palta et al., 2014; Sadanand et al., 2016) in T2D. Vincent & Hall (2015) confirmed the worst EF performance in several components such as inhibition, attention and working memory (WM). Pelimanni & Jehkonen (2019) in their meta-analysis confirmed deficits in processing speed, working memory and attention/concentration.

Recently, in a review of reviews as is titled their paper, Sola et al. (2024) concluded that T2D appears associated with cognitive decrements, with small effect sizes in the largest meta-analyses. Within this all literature, only a few studies have investigated the association between cognitive performance and C levels in older T2D people. Reynolds et al. (2010) found significant associations of higher fasting cortisol levels with working memory and poorer processing speed in a subsample of the Edinburgh Type 2 Diabetes Study, although they not included comparisons with healthy controls. In a 2-year prospective study, Chung et al. (2015) found an accelerated impairment of cerebral vasoregulation and a greater decline in cognitive function in the T2D participants in comparison to

controls. Then, more work is required to provide light on the association of HPA axis functioning and its impact on cognitive performance in T2D.

The main aim of the present study was to test whether the diurnal cortisol cycle was different between matched healthy and T2D older people, as well as if T2D people had a worst cognitive performance. Additionally, we wanted to analyse the relationships between two indexes of the diurnal cortisol cycle (CAR and DCS) and cognitive performance (DM, WM, and EF) in T2D people in comparison to controls. We expected that T2D group will have a flatter CAR (Bruehl et al., 2009; Panagiotou et al., 2021; Lederbogen et al., 2011) and DCS (Hackett et al., 2014; Lederbogen et al., 2011), in comparison to healthy people as well as worse cognitive performance in the domains examined. Moreover, based on previous studies in healthy individuals, we expected: i) a higher CAR to be associated with poorer DM performance but better WM and EF performance (Almela et al., 2012; Evans et al., 2012; ii) a flatter DCS to be associated with impairment in DM (O'Hara et al., 2007). We have no previous expectations about the association between WM and EF with DCS in T2D population. Finally, given the high prevalence and comorbidity in T2D (Zanovelli et al., 2016), we explored the potential role of depression in the relationships among cortisol indexes and cognitive functions, anticipating that depression would increase the strength of the relationship.

Methods

Participants

The final sample was composed of 51 T2D patients (26 men and 25 women) and 51 healthy participants (26 men and 25 women). For recruiting T2D subjects, informative advertisements were displayed on the primary care ambulatory and hospitals. Additionally, more information about the study and invitation to enrolment was purposed to these patients at their medical follow-ups. Non-diabetic-matched subjects belonged to a study program at the University of Valencia for people older than 55 years of age (see Table V.1 for sample characteristics).

All volunteers were excluded from participation when they presented the following criteria: smoking more than 10 cigarettes per day, abuse of alcohol or other drugs of abuse, psychiatric or neurological disorder, presence of cardiovascular disorder and visual or hearing impairment. None of the participants reported a stressful life event or had surgery under general anaesthesia during the past year. Individuals who were being medicated with drugs related to cognitive or emotional functions or medication that was able to influence hormonal levels (i.e., glucocorticoids, beta-blockers, antidepressants, asthma medication or thyroid therapies) or psychotropic substances, were also excluded. T2D participants had a doctor T2D diagnosed and all of them were medically treated. All the participants scored more than twenty-eight on the MMSE (Spanish version of the Mini-Mental Status Examination; Lobo et al., 1999), indicating the absence of cognitive impairment.

Table V.1 | Descriptive statistics (mean $\pm$ SEM) for total sample and both T2D and Healthy Groups.

	Total Sample (N=102)	T2D Group (N=51)	Healthy Group (N=51)	t/ χ^2	Sign. (p)
Age (years)	65.81 (.512)	65.92 (.758)	65.71 (.694)	.210	.834
BMI (kg/m ²)	28.57 (.451)	28.82 (.719)	28.33 (.558)	.545	.587
SES	5.29 (.122)	5.07 (.207)	5.49 (.135)	-1.713	.091
Glucose I	126.020 (5.008)	149.700 (6.204)	101.857 (6.267)	5.426	<.001
Glucose II	112.313 (3.563)	128.48 (4.468)	95.816 (4.513)	5.144	<.001
Δ Glucose	13.707(2.287)	21.220(3.939)	6.041(1.729)	3.529	<.001
Educational level (%)				6.734	.241
No studies	4.0	8.0	0		
Basic studies	35.6	36.0	35.3		
High school	31.7	32.0	31.4		
College or higher	13.9	21.6	23.3		
Marital status (%)				2.824	.420
Single	15.8	16.0	15.7		
Married	65.3	68.0	62.7		
Divorced	7.9	10.0	5.9		
Widowed	10.9	6.0	15.7		
AUCi	2.194 (.272)	1.478 (.362)	2.851 (.382)	-1.653	.102
CAR	4.485(5.334)	3.245(5.062)	5.627(5.371)	-1.561	.061
DCS	6.378 (.398)	6.377 (.607)	6.378 (.526)	-.478	.633
Awakening cortisol	8.552	8.403	8.687	-.481	.316
Bedtime cortisol	1.845	1.844	1.847	-.176	.430
Depression	7.461(.647)	10.92(.994)	4(.471)	6.292	<.001
Waist	107.222(1.208)	100.160(2.020)	95.216(1.705)	1.873	.064
Waist:Hip _{ratio}	.912(.008)	.928(.085)	.895(.083)	1.930	.056

Procedure and neuropsychological assessment

All participants received verbal and written information about the study and signed an informed consent form. The study was carried out according with the Declaration of Helsinki, and the Ethics Research Committee of the University of Valencia approved the protocol (1034878).

This study consisted of one individual session that started between 10:00 and 12:00 h. Before the session, the experimenter first checked whether all participants followed the instructions before participation: to refrain from heavy physical activity from the evening before the session, sleep as long as usual, and not consume alcohol since the night before the session. Additionally, they were instructed to drink only water, and not eat, smoke, take any stimulants (e.g., coffee, cola, tea, chocolate), or brush their teeth at least 1 h prior to the session.

In the session, participants performed a total of 6 tests that assessed different domains: DM, WM and EF. Two capillary blood samples were taken at the beginning and at the end of the session to measure glucose concentrations. Both groups followed the same time schedule for sample collection and questionnaires administered.

Declarative memory (DB) was assessed with the Spanish version (Miranda & Valencia, 1997) of the Rey Auditory Verbal Learning test (RAVLT) and The Story Recall subtest from the Spanish version (Alonso & Prieto, 2004) of the Rivermead Behavioural Memory Test (RBMT) (Wilson, Cockburn, & Baddeley, 1985). The RAVLT consists of reading a 15 wordlist during 5 consecutive trials, followed by a presentation of an interference list. After that, two recall trials are presented. The first is done after the recall of the interference list (immediate recall), and the second after a delay of 30 min (delayed recall). Three outcomes were used in the analyses: i) Total learning: total number of words referred across the five trials; ii) Immediate recall: number of words recalled after the interference trial; iii) Delayed recall: total number of words recalled after the 30-min delay. The RBMT subtest consists of reading aloud two brief stories by the experimenter. Then, the subjects had to recall as many “ideas” as possible just immediately

after their presentation (immediately recall), and after a 20-min delay (delayed recall). The total of the recalled “ideas” from the stories was calculated. Two outcomes were used for the analysis: i) Immediate Recall: sum of “ideas” referred from the two stories; ii) Delayed recall: sum of “ideas” referred from the two stories after 20 min of delay.

Working memory (WM) was evaluated with two verbal tests: Digit Span (DS) and Letter-Number Sequencing (LNS), from the Spanish version (Pereña et al., 2004) of the Wechsler Memory Scale III (Wechsler, 1997). The DS requires participants listen to a series of numbers (from 0 to 9) of increasing lengths (from two to nine digits). Participants must repeat the numbers in the same order (DS-Forward) and in reverse order (DS-Backward) in which they were presented. Each set length was tested twice. The task finalized when the participant failed the two consecutive trials of the same length. One point was given for each correctly repeated digit set. The maximum score possible is 16. Two outcomes were obtained: (i) DS Forward: the total number of correctly recalled attempts in the same order and (ii) DS Backward: the total number of correctly recalled attempts in the reverse order. The DS-Forward is a task used to measure the span component of WM and attention, and the DS-Backward was used to measure the executive component of WM (Conklin et al., 2000). For the LNS, the experimenter read aloud a sequence of alternating digits (from 0 to 9) and letters (from A to Z) of increasing length from 2 to 8 items. The participant had to repeat the series, ordering the digits in numerical order and the letters in alphabetical order. The test was finalized when the participant failed three consecutive trials of the same length. One point was given for each correctly recalled attempt. The maximum score possible is 16. The total number of correctly recalled attempts was obtained.

Executive Function (EF) Spanish versions of The Trail Making Test (TMT; Reitan, 1992; Fernández et al., 2002) and the Stroop Color-Word Test (SCWT; Golden, 1978; Peña-Casanova et al., 2009) were used to assess executive function. TMT is composed of two parts, TMT-A and TMT-B. In TMT-A, participant was instructed to connect circles numbered from 1 to 25 in numerical

sequence as quickly as possible. In TMT-B, the instruction was to alternate numbers from 1 to 13 and letters from A to L in ascending sequence. TMT-A was used to evaluate selective attention and processing speed, while attention-switching performance was assessed by the TMT-B. We obtained two outcomes: (i) TMT-A: the sum of the seconds required to finish the TMT-A, and (ii) TMT-B: the sum of seconds required to finish the TMT-B. The SCWT was administered to assess interference in 3 trials. Participants were required to read different tables as quickly as possible in 45 seconds. In the first trial, participants had to read color names written in black ink (W). In the second trial, participants had to name different color (C) patches in blue, green or red. In the third trial, participants had to name words-color (WC) printed in an inconsistent color ink (for example, the word green printed in red color). The WC' was calculated with the following formula: $WC' = (W \times C) / (W + C)$. Finally, we obtained the Stroop Interference (Interf: $WC - WC'$), which is a measure of the ability to inhibit automatic responses.

Salivary Cortisol

To measure the diurnal cortisol cycle, participants provided 4 saliva samples per day on 2 consecutive weekdays using salivettes (Sarstedt, Nümbrecht, Germany) at their home. The salivettes were stored in MEMS TrackCap containers (MEMS 6 TrackCap Monitor, Aardex Ltd. Switzerland) for recording the time the participants opened the container to provide a sample. Also, the subjects wrote the sampling times in a log. The saliva samples were provided immediately after awakening, 30- and 45-min post-awakening and immediately before bedtime. Participants stored their samples in the fridge, and they brought them to the university in less than 3 days after completion.

Once in the laboratory, the samples were centrifuged at 4000rpm for 15 min to obtain a clear supernatant that was stored at 80° C until the analyses were performed. Cortisol concentrations were determined by radioimmunoassay using the commercial kit Spectria Cortisol RIA from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/L., and the within- and inter-assay

variation coefficients were all below 8%. Each subject's samples were analyzed in the same trial.

Statistical analyses and data management

Cortisol values did not show normal distribution; therefore, they were log transformed. Because all cortisol samples correlated across the two days (all $p < .001$), the average cortisol levels for both days was used to calculate the following two cortisol indexes used in the analyses: i) CAR: to reflect the post-awakening measure of cortisol secretion was calculated by the cortisol area under the curve with respect to the increase (AUC_i, see Pruessner et al., 2003) from awakening, +30 and +45 min. Additionally, CAR was calculated by subtracting the awakening cortisol to the +30 min values, following the same procedure of Bruhl et al. (2009) and Hackett et al. (2014); this latter is named as CAR in the rest of manuscript; ii) DCS: to reflect the decline in cortisol levels during the day, and calculated by the awakening cortisol minus bedtime cortisol levels. A larger DCS value was interpreted as a steeper slope, reflecting a more diurnal decline, while a smaller DCS value was interpreted as a flatter slope, reflecting a less cortisol decline. Δ Glucose was calculated by subtracting glucose levels post session from the pre-stress levels. Higher Δ Glucose indicate higher glucose difference or change during the session.

Student t test and chi-square were used to test differences between groups (T2D and healthy) in socio-demographic variables, glucose levels, depression, cortisol indexes and neuropsychological tests (RAVLT total learning and learning slope, Digit Span, LNS and TMT). ANOVA for repeated measures was used to investigate group differences in cortisol levels during the day and DM (RAVLT and RBMT) performance, with Time (cortisol: Awakening, +30 min, +45 min, Bedtime; Glucose: pre and post; RAVLT: Trial 1, 2, 3, 4, 5, immediate and delay; RBMT: immediate and delayed), as the within-subject factor and Group (T2D and healthy) as a between-subject factor.

Moderation analyses were performed to test the associations between cortisol indexes and cognitive performance with Group (T2D vs healthy) or

Depression as moderators. At first, covariates were not included at the analyses (unadjusted analyses). Secondly, we repeated the analyses (adjusted analyses), for controlling cortisol and cognitive measurements. So, the following covariates were included: for CAR and AUCi, the time of awakening (Lederbogen et al., 2011; Hackett et al., 2014), and for the DCS, the time from awakening to bedtime (Hidalgo et al., 2021). We used only the main neuropsychological indexes for each test, and moderations were adjusted for each index if necessary. Specifically, RAVLT delayed and RBMT delayed were controlled for their immediate index, DS-Backward was controlled for DS-Forward and TMT-B was controlled for TMT-A. The PROCESS macro in SPSS (Model 1) was used, with 5,000 bootstrapped samples. We included CAR, AUCi, or DCS as independent variable, memory and EF performance as dependent variables and Group (T2D and healthy) or depression or as moderators.

For cortisol indexes, one healthy man was eliminated for being ± 3 SD from the mean. Six outliers were also eliminated for the neuropsychological analyses. Exactly, three women of the T2D group (one for RAVLT and TMT-A, one for TMT-A and one for TMT-B), one healthy woman for DS-Backward, and two healthy men for LNS.

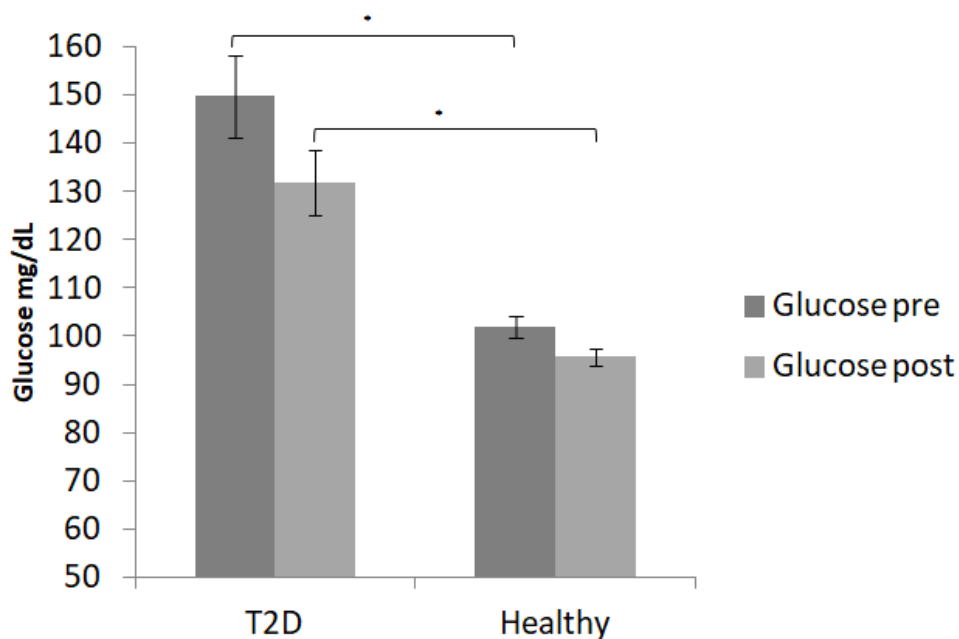
Greenhouse–Geisser was used when the requirement of sphericity in the analysis was violated. *Post hoc* planned comparisons were performed using Bonferroni adjustments for the *p* values. Statistical analyses were carried out using SPSS 28.0. All *p* values reported are two-tailed. The level of significance was fixed as <0.05 . For an easy interpretation, the values in the figures and tables represent raw values, and not logarithmic transformed values.

Results

Preliminary analyses

No significant differences between the T2D and healthy groups in age, SES, BMI, marital status, and education level were found (all $p > .091$). However, T2D patients had higher glucose levels pre and post session (both $p < .001$) than healthy participants and also higher Δ Glucose ($p < .001$) (Table V.1 and Figure V.1). There were significant differences between the two groups in depression ($p < .001$), and marginally in the Waist:Hip_{ratio} ($p = .056$) and Waist ($p = .064$). T2D had higher values in all cases than healthy participants (see Table V.1 and Figure V.1).

Figure V.1. Pre and post glucose levels for T2D and Healthy groups.



Note: Depicted values are means and error bars representing the SEM of glucose values (** $p \leq 0.001$, * $p \leq 0.05$).

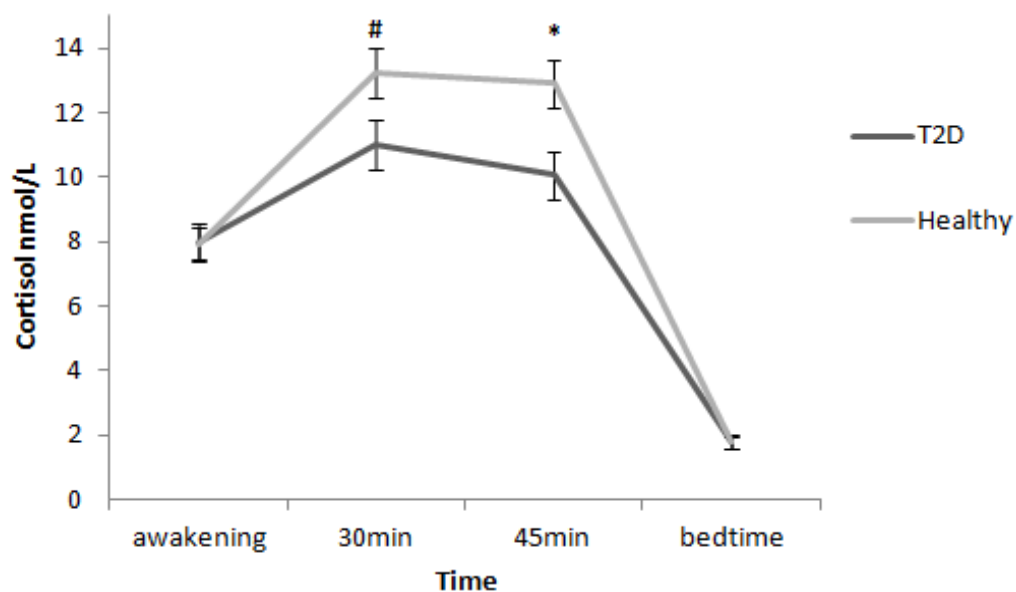
Cortisol pattern

Results showed a significant effect of Time ($F_{(2.018, 183.663)} = 543.283$, $p < .001$, $\eta^2_p = .857$), and marginally significant of the Time x Group interaction ($F_{(2.018, 183.663)} = 2.657$, $p = .072$, $\eta^2_p = .028$). *Post hoc* analyses revealed that overall participants' cortisol levels significantly increased from awakening to +30 min

($p < .001$), and then, cortisol levels started to decrease until bedtime (+30 vs 45 min: $p = .019$, and +45 vs bedtime: $p < .001$). In addition, T2D patients showed lower cortisol levels than healthy participants in both +30 min ($p = .051$) and +45 min ($p = .018$) samples. In T2D participants, cortisol levels significantly increased from awakening to +30 min ($p < .001$), and then, they started to decrease until bedtime (+30 vs 45 min: $p = .036$ and +45 vs bedtime: $p < .001$). In healthy participants, cortisol levels significantly increased from awakening to +30 min ($p < .001$) and later, remained stable (+30 vs 45 min: $p = .884$). After that, cortisol levels started to decrease until bedtime (+45 vs bedtime: $p < .001$) (Figure V.2).

Finally, no significant differences between the two groups were found in AUCi and DCS (both $p > .102$). A tendency towards significance was found in CAR, where T2D showed smaller increases from awakening to 30' ($p = .061$) (see Table V.1).

Figure V.2. Means and SEM of salivary cortisol concentrations



Note: Depicted values are means and error bars represent SEM of raw cortisol values. Significant differences between T2D and healthy (* p values ≤ 0.05 ; # trend towards significant differences).

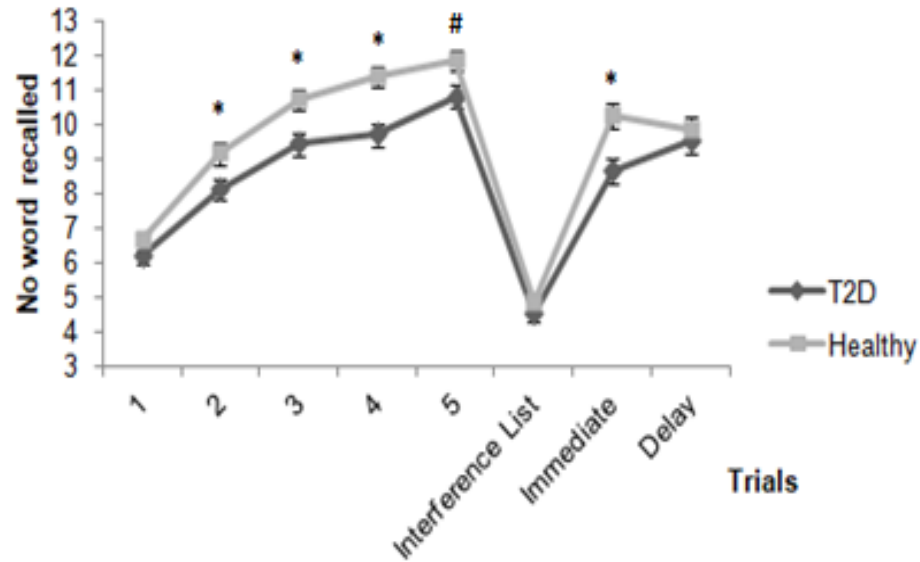
Cognitive performance

Declarative memory. The repeated measures ANOVA with RAVLT as the dependent variable showed significant effects of Trial ($F_{(4, 079, 395.632)} = 117.338$, $p < .001$, $\eta^2_p = .547$), Group ($F_{(1, 97)} = 4.994$, $p = .028$, $\eta^2_p = .049$), and the Trial x Group interaction ($F_{(4, 079, 395.632)} = 3.0545$, $p = .016$, $\eta^2_p = .031$). *Post hoc* analyses revealed that in both groups, there was a positive learning from trial 1 to trial 3 (all $p < .001$), a no significant increase from trial 3 to trial 4, ($p = .103$), and then a significant increase from trial 4 to 5 ($p < .001$). Fewer number of words were recalled in the trial immediately after the interference list (immediate recall) than in the trial before it (trial 5) ($p < .001$). In addition, all participants recalled a similar number of words in both the immediate and delayed recall trials ($p > .900$). Overall, T2D patients showed less recall than healthy participants ($p = .028$), and specifically in trials 2 ($p = .049$), 3 ($p = .015$), 4 ($p = .002$), 5 (as a trend, $p = .070$) and immediate recall ($p = .017$). No group differences were observed on trial 1 and delayed recall (both $p > .285$). T2D participants showed a positive learning from trial 1 to 3 (both $p < .001$). The number of words recalled were similar in the trials 3 and 4 ($p > 0.99$), although, a greater number of learned words was observed in the trial 4 in comparison to the trial 5 ($p < .001$). Significantly less words were learned in the immediate recall in contrast to trial 5 ($p < .001$). T2D participants were able to recall more words in the delayed trial than in the immediate trial ($p = .004$). Healthy participants showed a positive learning from trial 1 to trial 3, trials 1 vs 2, and 2 vs 3 (both $p < .001$). However, the numbers of words recalled were similar in the trials 3 and 4 ($p > 0.99$), and 4 and 5 ($p = .727$). Participants recalled lower number of words in the immediate recall in comparison to trial 5 ($p < .001$). No significant differences were observed between immediate and delayed recall ($p > 0.99$) (Figure V.3A).

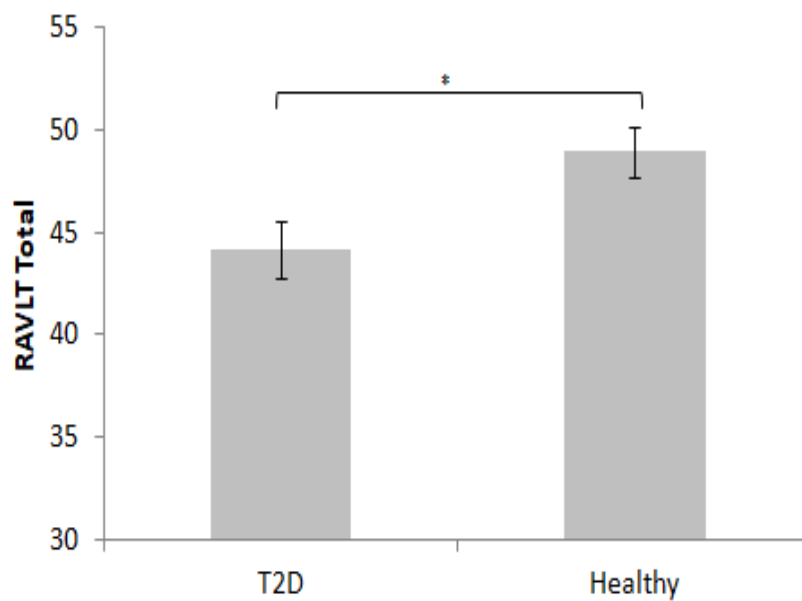
In addition, *t*-tests results showed significant group differences in total learning of the RAVLT ($t = -2.590$, $p = .011$). Participants in the T2D group presented worse total learning performance than those in the healthy group (T2D: $M = 44.200$; healthy: $M = 48.980$) (Figure V.3B).

Figure V.3. A) Means and SEM of the number of words recalled by the T2D and Healthy groups in each trial of the RAVLT. B) Means and SEM of the total learning.

A)



B)

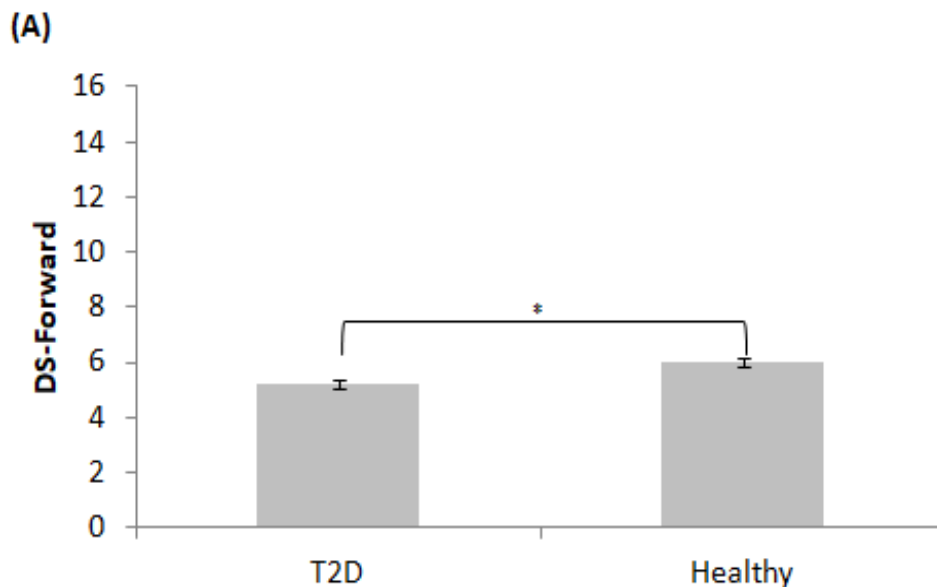


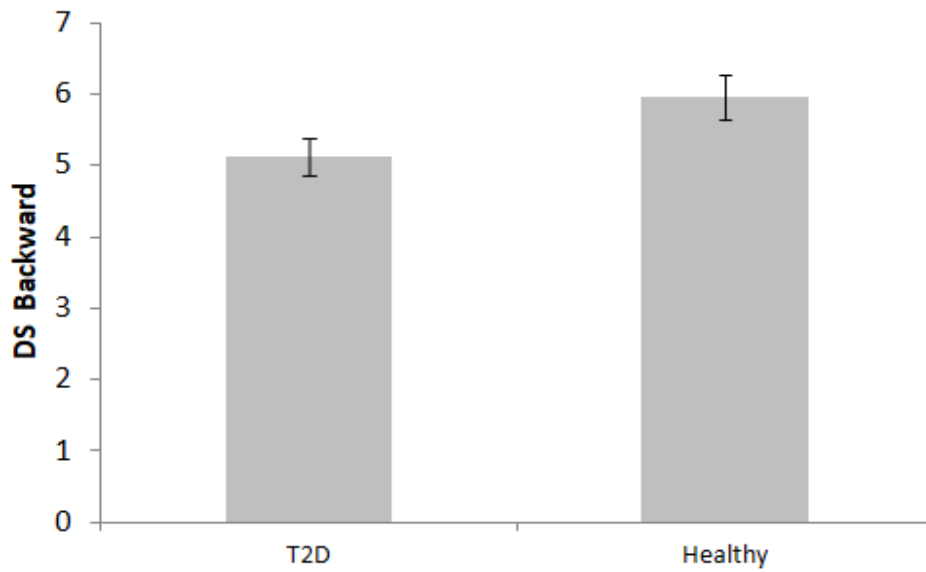
Note: Depicted values are means and error bars represent SEM of number of word recalled and total learning. Significant differences between T2D and healthy (** p values ≤ 0.001; * p values ≤ 0.05; # trend towards significant differences).

Regarding RBMT, an effect of Group ($F_{(1, 99)} = 8.046, p = .006, \eta^2_p = .075$) was found. Participants in the T2D group had lower recall than healthy participants. No significant differences were observed in Time ($F_{(1, 99.000)} = 2.047, p = .156, \eta^2_p = .020$) or in the Time x Group interaction ($F_{(1, 99)} = .003, p = .960, \eta^2_p = .001$).

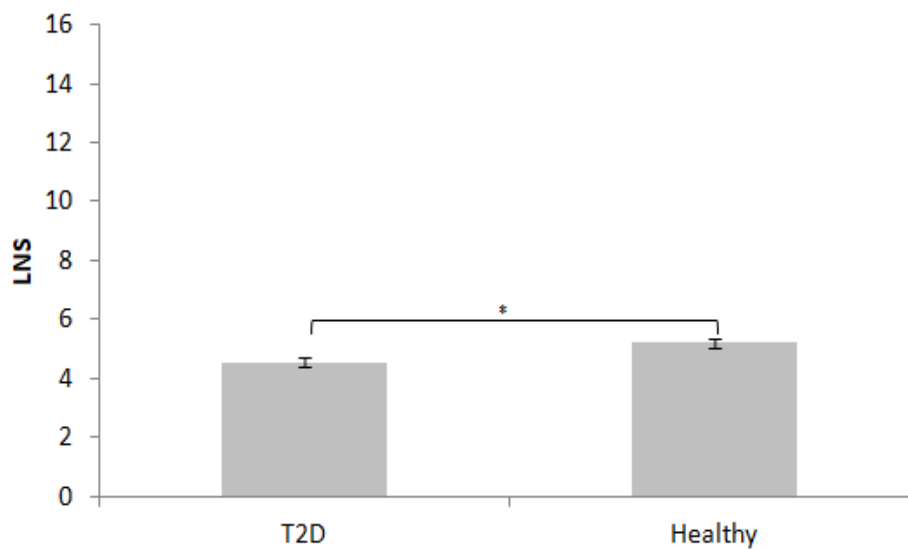
Working memory. Results of the *t*-test analyses revealed significant group differences on DS-Forward ($t = -2.958, p = .002$) and, marginally, on DS-Backward ($t = 1.826, p = .071$), showing T2D worse performance than healthy participants in both subtests (Figure V.4A). In addition, significant group differences were observed in LNS ($t = -2.708, p = .008$), showing T2D worse performance than healthy participants (Figure V.4B).

Figure V.4. A) Scores on DS -Forward (left) and DS-Backward (right). Values for DS-Backward are depicted for raw scores, *p* values correspond to adjusted data. **B)** Scores on Letter-Number sequencing (LNS) for T2D and Healthy groups. Depicted values are means and error bars represent the SEM.





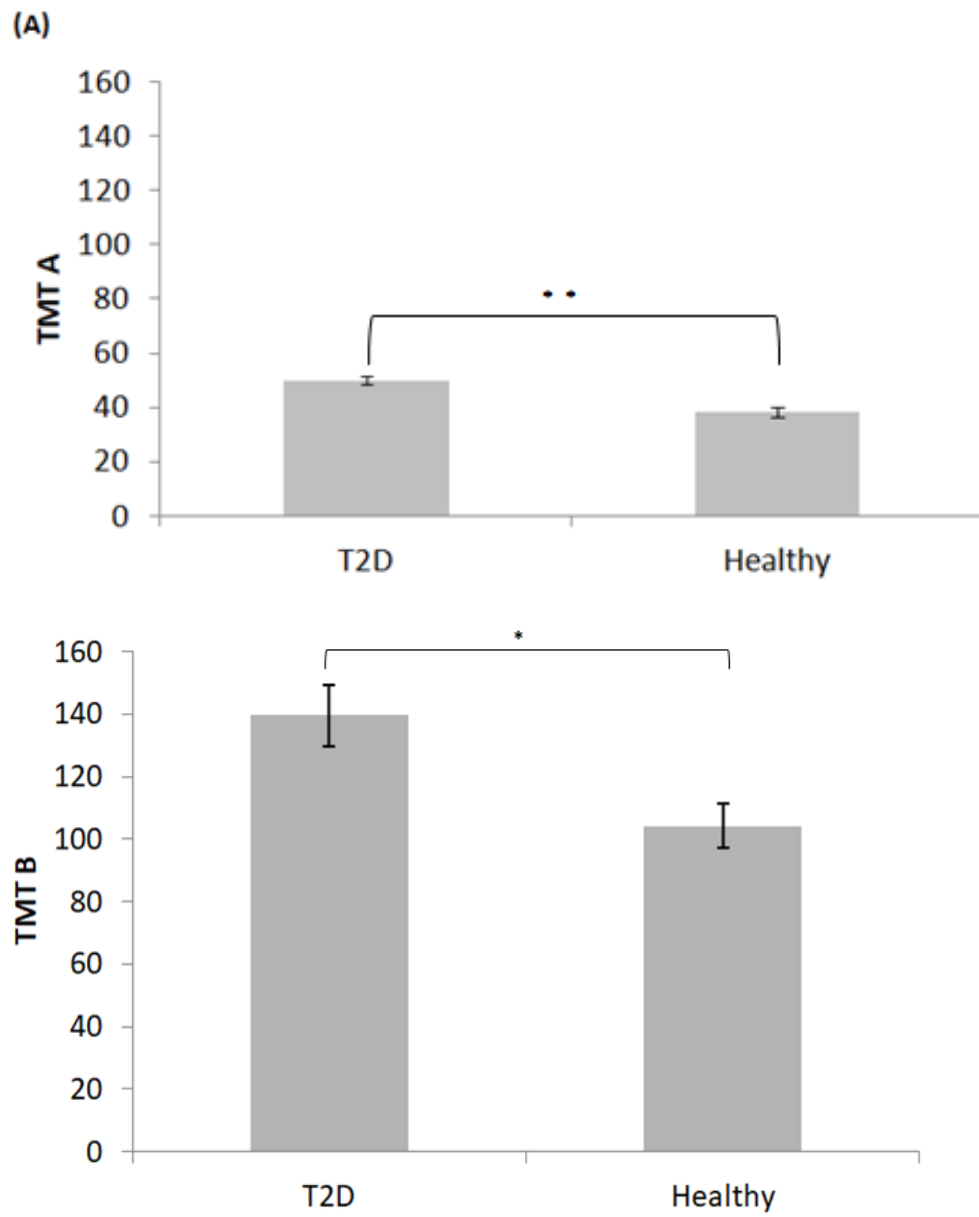
(B)



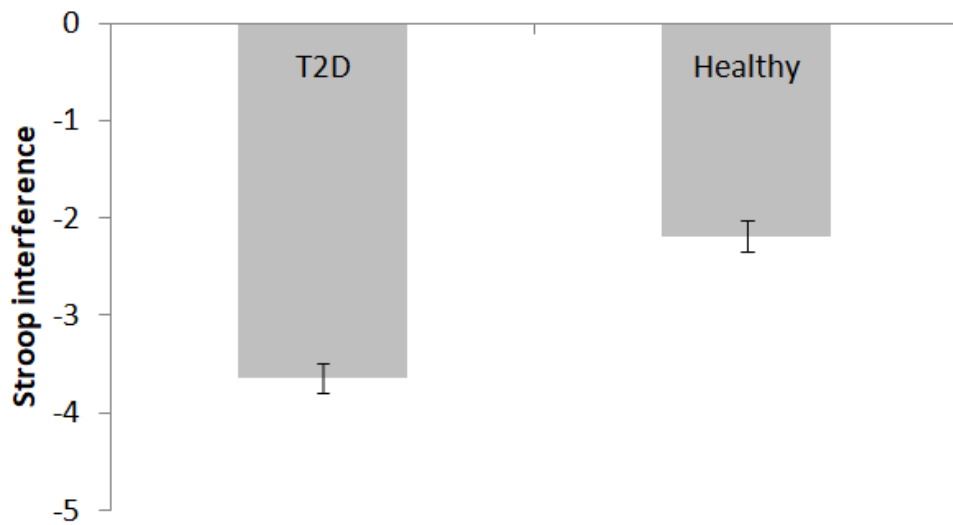
Note: Depicted values are means and error bars represent SEM * $p < 0.05$; ** $p \leq 0.001$.

Executive function. Group differences were found in TMT A ($t = 3.757$, $p < .001$) and in TMT B ($t = 2.974$, $p = .004$) (Figure V.5A). Thus, T2D participants had worse performance, with longer execution time on both tasks, than healthy participants. However, no significant differences were observed between groups in Stroop interference performance ($t = -.964$, $p = .338$) (Figure V.5B).

Figure V.5. Performance on TMT A and TMT B (A), and performance on Stroop interference (B) for T2D and Healthy group.



(B)



Note: Depicted values are means and error bars represent SEM * $p < 0.05$; ** $p \leq 0.001$

Relationship between cortisol indexes and cognitive performance

Moderation analyses between cortisol indexes and cognitive performance attending for group

For unadjusted analyses, results showed no AUCi X Group interaction (all $p > .417$). No relationship was found between AUCi and cognitive indexes neither in the T2D group (all $p > .290$) nor in the healthy group (all $p > .167$) (Table V.2). Adjusted analyses, despite not showing any significant AUCi X Group interaction (all $p > .338$), showed a significant negative relationship between AUCi and Stroop interference in the healthy group ($p = .044$) (Table V.3). Tables V.4 and V.5 depict unadjusted and adjusted for the CAR respectively. The only CAR X Group interaction was found for the Stroop interference model ($p = .049$), where a significant negative association was found for the healthy group ($p = .049$), but not for the T2D group ($p = .215$).

No interaction was found between DCS and Group in the unadjusted analyses (all $p > .110$). No significant relationship was found in any group between DCS and cognitive indexes (T2D group: all $p > .319$; healthy group: all $p > .115$) (Table V.6). After adjusting for covariates, no significant interaction between DCS and Group was found (all $p > .268$). There were not any significant relationships between DCS and cognitive indexes in any group (T2D group: all $p > .301$; healthy group: all $p > .235$) (Table V.7).

Table V.2 Conditional effect of AUCi on cognitive tests for T2D and healthy groups unadjusted

Moderator variable (W): groups (T2D and healthy)						
Independent variable (X): AUCi						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.001	$F = .102$		$p = .750$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.174	.163	1.065	.290	-.150	.498
Healthy	.107	.130	.819	.415	-.152	.366
Independent variable (X): AUCi						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.002	$F = .205$		$p = .652$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.104	.149	.695	.489	-.192	.399
Healthy	.007	.153	.045	.964	-.297	.311
Independent variable (X): AUCi						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.002	$F = .223$		$p = .638$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.075	.157	-.477	.635	-.386	.237
Healthy	.026	.144	.180	.858	-.260	.312
Independent variable (X): AUCi						
Dependent variable (Y): LNS						
ΔR^2 interaction =.007	$F = .665$		$p = .417$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.139	.167	-.831	.408	-.472	.193
Healthy	.048	.157	.306	.760	-.264	.360
Independent variable (X): AUCi						
Dependent variable (Y): TMT-B						
ΔR^2 interaction =.006	$F = .529$		$p = .469$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.144	.149	-.965	.337	-.441	.153
Healthy	.029	.186	.158	.875	-.340	.399

(Continue on next page)

Independent variable (X): AUCi

Dependent variable (Y): Stroop interference

ΔR^2 interaction = .001

$F = .064$

$p = .800$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
T2D	-.115	.187	-.612	.542	-.488	.258
Healthy	-.172	.123	-1.393	.167	-.417	.073

Note: AUCi = Area Under the Curve with respect to Increase; T2D = Type 2 diabetes; RAVLT delayed recall= Rey Auditory Verbal Learning Test; RBMT delayed recall = Rivermad Behavioural Memory Test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B.

Table V3 Conditional effect of AUCi on cognitive tests for T2D and healthy groups adjusted

Moderator variable (W): groups (T2D and healthy)						
Independent variable (X): AUCi						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.001	$F = .327$		$p = .569$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.026	.089	-.295	.768	-.204	.151
Healthy	.045	.090	.496	.621	-.134	.223
Independent variable (X): AUCi						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.001	$F = .031$		$p = .860$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.102	.085	1.203	.232	-.066	.271
Healthy	.081	.086	.941	.349	-.090	.252
Independent variable (X): AUCi						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.006	$F = .978$		$p = .325$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.104	.115	-.905	.360	-.333	.125
Healthy	.058	.119	.488	.627	-.178	.294
Independent variable (X): AUCi						
Dependent variable (Y): LNS						
ΔR^2 interaction =.012	$F = 1.179$		$p = .281$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.170	.147	-1.160	.249	-.462	.122
Healthy	.049	.142	.344	.732	-.234	.332
Independent variable (X): AUCi						
Dependent variable (Y): TMT-B						
ΔR^2 interaction =.002	$F = .260$		$p = .611$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.082	.128	-.644	.521	-.337	.172
Healthy	.007	.122	.056	.956	-.236	.250

(Continue on next page)

Independent variable (X): AUCi

Dependent variable (Y): Stroop interference

 ΔR^2 interaction = .014 $F = 1.376$ $p = .244$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
T2D	-.123	.149	-.824	.412	-.419	.174
Healthy	-.372	.153	-2.429	.017	-.677	-.067

Note: AUCi = Area Under the Curve with respect to Increase; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B.

Table V.4 Conditional effect of CAR on cognitive tests for T2D and healthy groups unadjusted

Moderator variable (W): groups (T2D and healthy)						
Independent variable (X): CAR						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.009	$F = .937$		$p = .336$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.001	.001	1.288	.201	.000	.001
Healthy	.146	.151	.968	.335	-.153	.445
Independent variable (X): CAR						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.001	$F = .002$		$p = .962$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.001	.000	-1.151	.253	-.001	.000
Healthy	-.007	.149	-.048	.961	-.304	.289
Independent variable (X): CAR						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.000	$F = .004$		$p = .953$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.000	.000	-1.105	.272	-.001	.000
Healthy	.009	.151	.059	.953	-.290	.308
Independent variable (X): CAR						
Dependent variable (Y): LNS						
ΔR^2 interaction =.003	$F = .028$		$p = .868$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.000	.000	-.287	.774	-.000	.000
Healthy	.023	.138	.166	.868	-.252	.298
Independent variable (X): CAR						
Dependent variable (Y): TMT-B						
ΔR^2 interaction =.001	$F = .073$		$p = .788$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.001	.000	1.250	.214	.000	.000
Healthy	.036	.134	.271	.787	-.229	.302

(Continue on next page)

Independent variable (X): CAR

Dependent variable (Y): Stroop interference

ΔR^2 interaction = .028

$F = 2.674$

$p = .106$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
T2D	.000	.000	-1.046	.298	-.000	.000
Healthy	-.267	.163	-1.636	.105	-.591	.057

Note: CAR = Cortisol awakening response; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B.

Table V.5 Conditional effect of CAR on cognitive tests for T2D and healthy groups adjusted

Moderator variable (W): groups (T2D and healthy)						
Independent variable (X): CAR						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.001	$F = .2795$		$p = .5983$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.001	.000	1.882	.063	.000	.002
Healthy	.046	.087	.529	.597	-.127	.219
Independent variable (X): CAR						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.004	$F = 1.134$		$p = .290$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.000	.000	-1.697	.093	-.001	.000
Healthy	.090	.085	1.064	.290	-.078	.258
Independent variable (X): CAR						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.000	$F = .007$		$p = .935$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.000	.000	.052	.959	-.000	.000
Healthy	.001	.118	.082	.935	-.225	.244
Independent variable (X): CAR						
Dependent variable (Y): LNS						
ΔR^2 interaction =.000	$F = .005$		$p = .943$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.000	.000	-.236	.814	-.000	.000
Healthy	.010	.141	.072	.943	-.271	.291
Independent variable (X): CAR						
Dependent variable (Y): TMT-B						
ΔR^2 interaction =.000	$F = .001$		$p = .973$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.000	.001	.739	.461	-.001	.000
Healthy	.004	.119	.034	.973	-.234	.242

(Continue on next page)

Independent variable (X): CAR

Dependent variable (Y): Stroop interference

 ΔR^2 interaction = .042 $F = 4.005$ $p = .049$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
T2D	.000	.000	1.249	.215	-.000	.000
Healthy	-.316	.158	-2.001	.049	-.629	-.002

Note: CAR = Cortisol awakening response; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B.

Table V.6 Conditional effect of DCS on cognitive tests for T2D and healthy groups unadjusted

Moderator variable (W): groups (T2D and healthy)						
Independent variable (X): DCS						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.010	$F = .896$		$p = .346$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.055	.160	-.345	.731	-.374	.263
Healthy	.140	.129	1.080	.283	-.117	.397
Independent variable (X): DCS						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.004	$F = .352$		$p = .555$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.074	.157	.474	.637	-.237	.386
Healthy	-.046	.127	-.358	.721	-.298	.207
Independent variable (X): DCS						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.001	$F = .045$		$p = .833$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.124	.160	.774	.441	-.194	.441
Healthy	.080	.131	.613	.542	-.179	.339
Independent variable (X): DCS						
Dependent variable (Y): LNS						
ΔR^2 interaction =.026	$F = 2.603$		$p = .110$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.128	.158	.808	.421	-.187	.443
Healthy	-.195	.122	-1.593	.115	-.438	.048
Independent variable (X): DCS						
Dependent variable (Y): TMT-B						
ΔR^2 interaction =.012	$F = .1.138$		$p = .289$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.165	.165	-1.002	.319	-.493	.162
Healthy	.058	.128	.450	.654	-.197	.312

(Continue on next page)

Independent variable (X): DCS

Dependent variable (Y): Stroop interference

ΔR^2 interaction = .007

$F = .577$

$p = .450$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
T2D	.068	.171	.399	.691	-.272	.409
Healthy	-.096	.133	-.725	.470	-.360	.168

Note: DCS = Diurnal Cortisol sSlope; T2D = Type 2 diabetes; RAVLT delayed recall= Rey Auditory Verbal Learning Test; RBMT delayed recall = Rivermad Behavioural Memory Test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B.

Table V.7 Conditional effect of DCS on cognitive tests for T2D and healthy groups adjusted

Moderator variable (W): groups (T2D and healthy)						
Independent variable (X): DCS						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.004	$F = 1.059$		$p = .306$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.034	.094	-.360	.720	-.222	.154
Healthy	.099	.089	1.110	.270	-.078	.276
Independent variable (X): DCS						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.001	$F = .034$		$p = .853$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.085	.091	.937	.352	-.095	.265
Healthy	.062	.086	.723	.472	-.108	.232
Independent variable (X): DCS						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.001	$F = .133$		$p = .716$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.078	.120	.651	.517	-.160	.316
Healthy	.018	.116	.153	.878	-.213	.248
Independent variable (X): DCS						
Dependent variable (Y): LNS						
ΔR^2 interaction =.001	$F = .130$		$p = .720$			
	Effect	SE	t	p	LLCI	ULCI
T2D	.061	.156	.388	.699	-.250	.371
Healthy	-.019	.159	-.118	.907	-.335	.298
Independent variable (X): DCS						
Dependent variable (Y): TMT-B						
ΔR^2 interaction =.008	$F = .843$		$p = .361$			
	Effect	SE	t	p	LLCI	ULCI
T2D	-.088	.136	-.648	.519	-.358	.182
Healthy	.081	.124	.657	.513	-.165	.327

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Independent variable (X): DCS

Dependent variable (Y): Stroop interference

ΔR^2 interaction = .006

$F = .498$

$p = .482$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
T2D	.083	.170	.489	.626	-.255	.421
Healthy	-.077	.151	-.502	.617	-.384	.229

Note: DCS = Diurnal Cortisol Slope; T2D = Type 2 diabetes; RAVLT delayed recall= Rey Auditory Verbal Learning Test; RBMT delayed recall = Rivermad Behavioural Memory Test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B.

Moderation analyses between cortisol indexes and cognitive performance attending for Depression

Unadjusted analyses showed no AUCi X Depression interaction (all $p > .152$). Despite this, a marginal significant interaction was found in the RBMT delayed recall model ($p = .089$), although the relationships were not significant for any of the levels of depression. In any of the models, no relationship was found between AUCi and cognitive indexes in any of the depression levels of low (all $p > .103$) or high depression (all $p > .198$). A marginal negative relationship was found for middle levels of depression, where higher AUCi was related to lower Stroop interference ($p = .085$) (Table V.8). For adjusted analyses, a significant marginal interaction was found between AUCi X Depression in the RAVLT delayed recall model (all $p > .071$). However, this relationship between AUCi and RAVLT delayed recall was not significant at any of the depression levels (low: $p = .229$; middle: $p = .549$; high: $p = .144$). Although there was no founding a significant interaction AUCi X Depression in the Stroop Interference model ($p = .441$), a negative significant relationship was found between AUCi and Stroop interference for low ($p = .049$), and middle levels of depression ($p = .029$). No other AUCi X Depression interactions were found (all $p > .188$) (Table V.9). Similar results were found for the CAR (Tables V.10 and V.11).

Table V.8 Conditional effect of AUCi on cognitive tests for different depression levels unadjusted

Moderator variable (W): Depression						
Independent variable (X): AUCi						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.004	$F = .409$		$p = .524$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.051	.152	.338	.736	-.249	.353
-.377	.093	.114	.816	.417	-.133	.318
.848	.203	.164	1.237	.219	-.123	.529
Independent variable (X): AUCi						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.029	$F = 2.940$		$p = .089$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.240	.145	1.648	.103	-.049	.529
-.377	.133	.109	1.226	.223	-.083	.350
.848	-.150	.157	-.956	.341	-.461	.161
Independent variable (X): AUCi						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.001	$F = .018$		$p = .894$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	-.010	.152	-.068	.946	-.311	.291
-.377	-.019	.114	-.165	.869	-.245	.207
.848	-.042	.161	-.258	.797	-.362	.279
Independent variable (X): AUCi						
Dependent variable (Y): LNS						
ΔR^2 interaction =.020	$F = 1.899$		$p = .172$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.105	.141	.741	.460	-.176	.385
-.377	.022	.106	.212	.833	-.189	.234
.848	-.197	.152	-1.297	.198	-.498	.105

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Independent variable (X): AUCi**Dependent variable (Y): TMT-B** ΔR^2 interaction = .020 $F = 2.088$ $p = .152$

BDI	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	-.195	.132	-1.472	.144	-.457	.068
-.377	-.114	.099	-1.138	.258	-.312	.085
.848	.102	.144	.709	.480	-.183	.387

Independent variable (X): AUCi**Dependent variable (Y): Stroop interference** ΔR^2 interaction = .006 $F = .540$ $p = .464$

BDI	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	-.265	.164	-1.611	.111	-.591	.062
-.377	-.215	.124	-1.741	.085	-.461	.031
.848	-.084	.167	-.502	.617	-.417	.249

Note: AUCi = Area Under the Curve with respect to Increase; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test ; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B; BDI = Beck Depression Inventory; values for quantitative moderators are the 16th, 50th and 84th percentiles.

Table V.9 Conditional effect of AUCi on cognitive tests for diferent depression levels adjusted

Moderator variable (W): Depression						
Independent variable (X): AUCi						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.012	$F = 3.348$		$p = .071$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	-.112	.093	-1.212	.229	-.297	.072
-.377	-.042	.070	-.601	.549	-.182	.098
.848	.144	.098	1.475	.144	-.050	.339
Independent variable (X): AUCi						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction = .001	$F = .052$		$p = .820$			
	Effect	SE	t	p	LLCI	ULCI
-.836	.102	.086	1.181	.241	-.070	.274
-.377	.094	.065	1.446	.152	-.035	.222
.848	.071	.092	.773	.441	-.112	.255
Independent variable (X): AUCi						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.007	$F = 1.042$		$p = .310$			
	Effect	SE	t	p	LLCI	ULCI
-.836	-.147	.121	-1.218	.227	-.388	.093
-.377	-.096	.091	-1.052	.296	-.277	.085
.848	.041	.127	.323	.747	-.212	.295
Independent variable (X): AUCi						
Dependent variable (Y): LNS						
ΔR^2 interaction =.018	$F = 1.750$		$p = .188$			
	Effect	SE	t	p	LLCI	ULCI
-.836	.073	.144	.505	.615	-.214	.359
-.377	-.006	.109	-.058	.954	-.224	.211
.848	-.217	.153	-1.424	.158	-.521	.086

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Independent variable (X): AUCi

Dependent variable (Y): TMT-B

ΔR^2 interaction = .005

$F = .542$

$p = .463$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	-.103	.124	-.833	.407	-.350	.143
-.377	-.065	.094	-.692	.491	-.252	.122
.848	.036	.133	.274	.784	-.228	.301

Independent variable (X): AUCi

Dependent variable (Y): Stroop interference

ΔR^2 interaction = .006

$F = .598$

$p = .441$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	-.325	.162	-2.002	.049	-.647	-.002
-.377	-.274	.123	-2.224	.029	-.519	-.029
.848	-.139	.165	-.845	.400	-.468	.189

Note: : AUCi = Area Under the Curve with respect to Increase; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test ; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B; BDI = Beck Depression Inventory; values for quantitative moderators are the 16th, 50th and 84th percentiles.

Table V.10 Conditional effect of CAR on cognitive tests for diferent depression levels unadjusted

Moderator variable (W): Depression						
Independent variable (X): CAR						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.005	$F = .491$		$p = .485$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.000	.000	-.026	.979	-.000	.000
-.377	.000	.000	.287	.774	-.000	.000
.848	.000	.000	1.555	.123	.000	.000
Independent variable (X): CAR						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.012	$F = 1.272$		$p = .262$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.000	.000	.319	.750	-.000	.000
-.377	.000	.000	.115	.908	-.000	.000
.977	.000	.000	-.873	.385	-.000	.000
Independent variable (X): CAR						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.001	$F = .060$		$p = .807$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.000	.000	-.324	.746	-.000	.000
-.377	.000	.000	-.434	.665	-.000	.000
.848	.000	.000	-.817	.416	-.000	.000
Independent variable (X): CAR						
Dependent variable (Y): LNS						
ΔR^2 interaction =.008	$F = .832$		$p = .364$			
BDI	Effect	SE	T	p	LLCI	ULCI
-.836	.000	.000	.606	.546	-.000	.000
-.377	.000	.000	.426	.671	-.000	.000
.848	-.000	.000	-.943	.348	-.000	.000

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Independent variable (X): CAR**Dependent variable (Y): TMT-B** ΔR^2 interaction = .004 $F = .458$ $p = .500$

BDI	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.000	.000	.028	.978	-.000	.000
-.377	.000	.000	.187	.852	-.000	.000
.872	.000	.000	.821	.413	.000	.000

Independent variable (X): CAR**Dependent variable (Y): Stroop interference** ΔR^2 interaction = .000 $F = .012$ $p = .911$

BDI	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.000	.000	-.463	.645	-.000	.000
-.377	.000	.000	-.565	.573	-.000	.000
.995	.000	.000	-.867	.388	-.000	.000

Note: CAR = Cortisol Awakening Response; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test ; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B; BDI = Beck Depression Inventory; values for quantitative moderators are the 16th, 50th and 84th percentiles.

Table V.11 Conditional effect of CAR on cognitive tests for diferent depression levels adjusted

Moderator variable (W): Depression						
Independent variable (X): CAR						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.004	$F = 1.215$		$p = .273$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.000	.000	-.075	.941	-.000	.000
-.377	.000	.000	.356	.723	-.000	.000
.848	.000	.000	2.506	.014	.000	.000
Independent variable (X): CAR						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.010	$F = 3.326$		$p = .071$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.000	.000	.971	.334	-.000	.000
-.377	.000	.000	.568	.571	-.000	.000
.848	-.000	.000	-2.331	.022	-.000	.000
Independent variable (X): CAR						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.000	$F = .006$		$p = .941$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.000	.000	.129	.897	-.000	.000
-.377	.000	.000	.145	.884	-.000	.000
.848	.000	.000	.099	.920	-.000	.000
Independent variable (X): CAR						
Dependent variable (Y): LNS						
ΔR^2 interaction =.009	$F = .975$		$p = .326$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.000	.000	.767	.445	-.000	.000
-.377	.000	.000	.636	.526	-.000	.000
.848	-.000	.000	-.734	.465	-.000	.000

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Independent variable (X): CAR**Dependent variable (Y): TMT-B** ΔR^2 interaction = .003 $F = .375$ $p = .542$

BDI	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.000	.000	-.256	.796	-.000	.000
-.377	.000	.000	-.101	.919	-.000	.000
.848	.000	.000	.932	.354	-.000	.000

Independent variable (X): CAR**Dependent variable (Y): Stroop interference** ΔR^2 interaction = .006 $F = .534$ $p = .467$

BDI	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.000	.000	1.202	.233	-.000	.000
-.377	.000	.000	1.336	.185	-.000	.000
.848	.000	.000	.727	.469	-.000	.000

Note: CAR = Cortisol Awakening Response; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B; BDI = Beck Depression Inventory; values for quantitative moderators are the 16th, 50th and 84th percentiles.

A significant DCS X Depression interaction was found in the RAVLT delayed recall model unadjusted analyses ($p = .025$). A significant positive relationship was found between DCS and RAVLT delayed recall for low depression levels ($p = .037$). No other DCS X Depression interaction was found for unadjusted analyses (all $p > .195$) (Table V.12). For adjusted analyses, a significant DCS X Depression interaction was found in the RAVLT delayed recall model ($p = .028$). A marginal positive significant relationship was found between DCS and RAVLT delayed recall for low depression levels ($p = .054$) (Table V.13).

Table V.12 Conditional effect of DCS on cognitive tests for diferent depression levels unadjusted

Moderator variable (W): Depression						
Independent variable (X): DCS						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=.053	$F = 5.231$		$p = .025$			
BDI	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.282	.133	2.112	.037	.017	.548
-.377	.124	.108	1.145	.255	-.091	.339
.848	-.297	.206	-1.445	.152	-.705	.111
Independent variable (X): DCS						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.005	$F = .454$		$p = .502$			
	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.028	.132	.210	.834	-.234	.289
-.377	.073	.107	.680	.498	-.140	.286
.848	.194	.200	.968	.336	-.204	.591
Independent variable (X): DCS						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.002	$F = .182$		$p = .671$			
	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.064	.131	.487	.627	-.196	.323
-.377	.035	.107	.329	.742	-.177	.248
.867	-.041	.202	-.205	.838	-.442	.359
Independent variable (X): DCS						
Dependent variable (Y): LNS						
ΔR^2 interaction =.006	$F = .572$		$p = .451$			
	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.105	.145	.724	.471	-.183	.392
-.377	.051	.113	.454	.651	-.173	.275
.848	-.091	.199	-.459	.647	-.487	.304

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Independent variable (X): DCS**Dependent variable (Y): TMT-B** ΔR^2 interaction = .017 $F = 1.707$ $p = .195$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.075	.119	.631	.529	-.161	.311
-.377	-.005	.098	-.049	.961	-.199	.190
.848	-.217	.184	-1.179	.242	-.584	.149

Independent variable (X): DCS**Dependent variable (Y): Stroop interference** ΔR^2 interaction = .015 $F = 1.292$ $p = .259$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	-.131	.144	-.908	.367	-.417	.156
-.377	-.047	.117	-.403	.688	-.280	.185
.848	.175	.218	.803	.424	-.259	.609

Note: DCS = Diurnal cortisol slope; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B; BDI = Beck Depression Inventory; values for quantitative moderators are the 16th, 50th and 84th percentiles.

Table V.13 Conditional effect of DCS on cognitive tests for diferent depression levels adjusted

Moderator variable (W): Depression						
Independent variable (X): DCS						
Dependent variable (Y): RAVLT delayed recall						
ΔR^2 interaction=. 018	$F = 4.984$		$p = .028$			
BDI	Effect	SE	t	p	LLCI	ULCI
-.836	.156	.080	1.947	.054	-.003	.316
-.377	.062	.065	.951	.344	-.068	.193
.848	-.188	.126	-1.490	.140	-.439	.063
Independent variable (X): DCS						
Dependent variable (Y): RBMT delayed recall						
ΔR^2 interaction =.001	$F = .021$		$p = .886$			
	Effect	SE	t	p	LLCI	ULCI
-.836	.066	.075	.876	.383	-.084	.215
-.377	.071	.061	1.163	.248	-.051	.193
.848	.086	.118	.734	.465	-.148	.321
Independent variable (X): DCS						
Dependent variable (Y): DS-Backward						
ΔR^2 interaction =.011	$F = 1.583$		$p = .212$			
	Effect	SE	T	p	LLCI	ULCI
-.836	.115	.101	1.129	.262	-.087	.316
-.377	.048	.084	.572	.569	-.119	.215
.848	-.130	.160	-.808	.421	-.448	.189
Independent variable (X): DCS						
Dependent variable (Y): LNS						
ΔR^2 interaction =.009	$F = .920$		$p = .340$			
	Effect	SE	T	p	LLCI	ULCI
-.836	.108	.144	.753	.453	-.178	.395
-.377	.040	.113	.357	.7222	-.184	.264
.848	-.142	.202	-.704	.483	-.543	.259

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Independent variable (X): DCS**Dependent variable (Y): TMT-B** ΔR^2 interaction = .014 $F = 1.594$ $p = .210$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	.089	.108	.827	.410	-.125	.304
-.377	.017	.089	.194	.847	-.160	.195
.848	-.175	.173	-1.010	.315	-.518	.169

Independent variable (X): DCS**Dependent variable (Y): Stroop interference** ΔR^2 interaction = .104 $F = 1.206$ $p = .275$

	Effect	SE	<i>t</i>	<i>p</i>	LLCI	ULCI
-.836	-.131	.145	-.903	.369	-.419	.157
-.377	-.049	.118	-.409	.684	-.284	.187
.848	.171	.225	.761	.449	-.276	.618

Note: DCS = Diurnal cortisol slope; T2D = Type 2 diabetes; RAVLT delayed recall= Rey auditory verbal Learning test; RBMT delayed recall = Rivermad Behavioural Memory test delayed recall; DS-Backward = Digit Span Backward; LNS= Letter-number sequencing; TMT-B= Trail Making Test B; BDI = Beck Depression Inventory; values for quantitative moderators are the 16th, 50th and 84th percentiles.

Discussion

In this study we aimed to analyse differences between T2D and healthy people in several indexes of the diurnal cortisol cycle and in some cognitive domains as well in their relationships. Regarding HPA axis, differences between T2D and healthy participants were found at +30 min and +45 min after awakening, having T2D lower C levels than healthy participants, but not in the CAR and DCS, indexes of the cortisol diurnal cycle. However, significant differences between both groups were found in DM, WM and EF although not in the Stroop interference. The moderation analyses showed only a significant negative relationship between CAR and Stroop in healthy controls after controlling for covariates. Since there were significant differences in depression between T2D and controls, we carried out also moderation analyses taking in account depression as moderating factor. These latter analyses showed significant and negative relationships between CAR and Stroop interference for low and middle depression scores, although the CAR X Depression interaction was not statistically significant. Finally, we found a significant DCS X Depression interaction in non and in adjusted models in the RAVLT delayed recall model, with a positive relationship between DCS and RAVLT delayed recall for low depression levels.

Differences in cortisol diurnal cycle

As mentioned, we do not find significant differences in CAR and DCS among T2D and healthy people. A flatter CAR has mostly been reported (Bruehl et al., 2009; Lederbogen et al., 2011; Panagiotou et al. (2021), or not significant differences (Hackett et al., 2014; Johar et al., 2016). Thus, Lederbogen et al. (2011) reported a significant lower CAR in diabetic people from a sample of KORA (Cooperative Health Research in the Region of Augsburg)-AGE study, calculated by subtracting the awakening from the 30 min value; however, with a different sample of this same program not significant differences were found afterwards (Johar et al., 2016). Also, Bruehl et al. (2009) described lower 30 min C values as well as a

flattened CAR. Panagiotou et al. (2021) reported a flattened CAR as the difference between T0 and 30 min. In our study, T2D patients showed lower cortisol levels than healthy participants at +30 min and +45 min, which coincides with lower C levels at 30 min, that is, we did not find the expected peak found in healthy people. In all mentioned studies, the CAR was calculated on two sampling points (on awakening and 30 min after awakening) and only a day, when it is recommended to assess CAR with a minimum of 3 saliva samples in at least two days (waking, 30 min and 45 min after waking) (Stalder et al., 2016). This could contribute to the different results.

Regarding DCS, we did not observe differences between groups. Our results are similar than those of Bruehl et al. (2009). In contrast, Lederbogen et al. (2011) reported a trend to a smaller slope whereas Hackett et al. (2014) observed a flattened DCS in participants with T2D; in this latter case, authors attributed this difference more to higher levels at bedtime than to lower levels at awakening. Again, differences in the way to calculate the DCS could contribute to the heterogeneous results. Interestingly, in our case we did not find significant differences between groups in the awakening and bedtime C values.

Differences in cognitive assessment

In order to assess the possible cognitive differences between T2D and healthy older people, we assessed DM, WM and EF, through several neuropsychological tests. In their review, Palta et al. (2014) concluded that the more sensitive tests to assess the largest performance differential for T2D persons relative to controls were RAVLT, Digit Span, TMT and Stroop, that showed the largest effect sizes. We employed them together a second test for each cognitive domain.

Thus, we evaluated DM by means of RAVLT and RBMT. While in the RAVLT the participants had to remember an independent list of words, in the RBMT they have to recall paragraphs. We found significant worse performance of T2D people than controls on the total learning score and also in the immediate recall of the RAVLT, but not in delayed recall. This coincides with results of the meta-analyses of Palta et al. (2014) and Sadanand et al. (2016) who indicated more sensitivity in the immediate compared to the delayed recall.

With respect of the RBMT, we found a significant worse performance in T2D patients but not differences in immediate and delayed memory. Previously Shimada et al. (2010) employing this test did not find significant differences between T2D and controls. Our results go in line with the Sadanand et al.' (2016) conclusions about both immediate and delayed recall in paragraph tests exhibited small differences. The RAVLT is an unstructured verbal memory list-learning task which requires greater cognitive demands for learning, encoding, and retrieval than structured tasks such as RBMT or others which provides semantic contexts (Palta et al., 2014). According to Lezak et al. (2004), whereas a word list learning test requires greater involvement of prefrontal functioning, a paragraph recall test is considered to be the clearest measure of DM, dependent of the hippocampal functioning.

Regarding WM, our results revealed worse performance in T2D than in healthy people in DS-Forward index and LNS, and marginal differences in DS-Backward. Also, Bruehl et al. (2007) did not find differences in DS-Backward. However, Mollon et al. (2020) reported lowest performance in DS-Backward and LNS in a T2D subsample of the San Antonio Family Heart Study in comparison to unaffected people of the same program and suggested an overlap between the genetic factors influencing T2D and performance on measures of attention and working memory among others. Mallorquí-Ballé et al. (2018), analysing basal data from the PREDIMED-plus study, found no differences between T2D and controls in both subtests of Digit Span (DS). In our case, we found significant differences in the second test employed, LNS test, which suppose more demands even than DS-Backward, since implies manipulation of more information at very short time period. In general, deficits in WM in T2D are accepted although with some heterogeneous results. Pelimanni and Jehkonen (2018) indicated that WM was among cognitive domains with more differences found in middle age, under 65, although not all meta-analyses agree (Palta et al., 2014). These latter authors also emphasised the importance of age for the differential cognitive effects of T2D. This aspect has also been highlighted more recently by Antal et al. (2022) who suggest that the neurocognitive impact of T2D implies a marked acceleration of normal brain aging.

Regarding the EF, worse performance in TMT A and TMT B was found, confirming previously reported results (Palta et al., 2014; Vincent & Hall, 2015; Mansur et al., 2018). However, we did not observe differences in the Stroop interference test between groups in line with Saczynski et al. (2008) but in contrast with that reported in the meta-analyses of Palta et al. (2014) and Vincent & Hall (2015). Again, some differences in how variables are analysed could contribute to explain these discrepancies. In some studies results are obtained directly from the Part III (Word-color interference), but in others are calculated by subtractions of the Stroop II from the Stroop III subtest scores (Murray et al., 2011) or with specific formulas (Montoliu et al., 2018).

Finally, it is worth noting that results obtained in DM and EF deficits are generally with T2D patients with other renal or cardiovascular diseases (for instance, Murray et al., 2011). For all this, the need to take in account different biomarkers and cognitive tests to detect early dysfunctions that be able to prevent the future impairments have been emphasized (Bruce et al., 2008).

As mentioned above, only a few studies have investigated how the CAR and DCS are associated with cognitive performance in T2D compared with healthy participants. We did not find any significant relationships in T2D group although a significant negative relationship between CAR and Stroop interference in the healthy group was found. In this sense, there is some evidence that a larger CAR is associated with improvements in cognition at older ages (Gardner et al., 2019). These results contrast with those found by O'Hara et al. (2007) in community-dwelling older adults participating in an investigation on cognitive decline although without T2D diagnosis.

The HPA dysregulation has been associated with T2D together with other noncommunicable diseases (NCDs), that represent an emerging global health threat and require early detection and prevention (Jones and Gwenin, 2020). In spite of the cognitive deficits associated to T2D as well as certain dysregulation of the functioning of the HPA axis, relationships between both dimensions were not found in this study employing a small but strictly matched sex-mixed T2D

sample with healthy controls. In the literature, most of the positive results reported on these relationships are from epidemiological studies with larger number of subjects but also sometimes less precise control of confounding factors.

Since diabetes is a chronic metabolic stressor, this has led to the suggestion that common mechanistic mediators are responsible for neuroplasticity deficits in both diabetes and depression. Similar neurochemical and anatomical basis for cognitive impairments in diabetes with those exhibited as consequence of chronic stress and stress-related psychiatric illnesses have been suggested (Reagan, 2012). Clearly, T2D people have more risk of depression compared to non-diabetic persons (Anderson et al., 2001). Depression increases the risk for progressive insulin resistance and incident T2D although the nature of this relation is not completely understood. On the one hand, depression may result from difficulties in coping with chronic disease, but, on the other hand, metabolic consequences of this disease may predispose people to depression or result from cerebral vascular damage. In addition, the relation may be bidirectional, as depression may even predispose the development of T2D (Reijmer et al., 2010). And as we found significant differences between T2D and controls in depressive symptoms that can affect cognitive performance, we explored if they could affect it in our study. Thus, a second block of moderation analyses were carried out taking in account scores in depression. A negative relationship between CAR and Stroop interference for low and middle levels of depression while a positive relationship between DCS and RAVLT delayed recall for low depression levels were found. These findings suggest that depression moderates the relationships between cortisol indexes and certain cognitive domains studied. The results regarding depressive symptomatology should be replicated in larger studies with samples that exhibit higher depressive scores, as our sample showed subclinical levels of depression. This limitation may explain why significant results were found only for low to moderate levels.

Conclusions

In sum, we found significant worse performance in memory, both dependent on hippocampus and PFC, although not in all tests employed. It is known that the EF has been linked to worse adherence to medical treatment, worse dietary control in older people and functional independence. Therefore, further studies should be conducted to screen for EF impairments and helping patients with their self-care and reduce the problems associated with the disease.

Chapter 6

General Discussion

The purposes of this doctoral thesis were to investigate the psychological, the SNS and the HPA-axis responses to an acute psychosocial stressor and the impact of this stressor on working and declarative memories in older T2D people. In addition, the HPA-axis functioning in basal situations (non-stress), the cognitive performance and the relationship between them were also investigated in this population. Specifically, the first study assessed the psychobiological (i.e., mood, sAA, and C) response to an acute stressor (i.e., TSST), and its effect on WM performance in diagnosed and medically treated older people with T2D. The second study examined the psychobiological (i.e., affect, sAA and salivary cortisol) response and the impact of acute stress on declarative memory retrieval performance in T2D older people in comparison to a group of age and sex matched healthy participants. Finally, the third study investigated the differences between T2D older and healthy older adults in the diurnal cortisol cycle, the cognitive performance and the relationships between the diurnal cortisol cycle and the cognitive performance.

The results have been detailed in the previous chapters. In this chapter, a brief discussion of the main results is presented, and clinical implications, limitations, and future directions arising from the results obtained are addressed.

Summary of the main findings

Study 1

Previous literature has shown that psychological stress has been identified as a risk factor for the onset and progression of many diseases. Specifically, it has been considered that stress plays a significant role in the etiology (Surwit et al., 1992), and T2D development (Heraclides et al., 2009; Eriksson et al., 2013; Hackett and Steptoe, 2016). The aim of this first study was to determine the response to an acute psychosocial stressor and its effects on WM in 50 older T2D participants between 52 to 77 years. Psychological assessment was conducted to test the participants' self-efficacy, situational appraisal and mood (PANAS).

The individuals were assigned to a stress or control condition. Acute stress was elicited by the TSST (Kirschbaum et al., 1993), and the physiological outcomes (sAA and C), and mood were analysed as measures of stress response. Results showed that the stress task required more effort and was perceived as more difficult, frustrating, and stressful than control task.

Furthermore, the TSST elicited mood changes characterized by decreases in positive mood and increases in negative mood. The main results of our study are consistent with previous literature in healthy older adults (Almela et al., 2011; Hidalgo et al., 2020) confirming that a psychosocial stressor (i.e., TSST) generates a similar mood response in T2D patients than in healthy older adults.

In accordance with our hypotheses, the TSST induced a cortisol response in T2D participants. Thus, our results replicate a previous study carried out by Faulenbach et al. (2011) reporting a C increase in T2D older adults 30 min after the TSST. In addition, in the Faulenbach's study, the C increase was greater in the fasting state coinciding with our study where participants were in a fasting state for between 1 and 2 hours.

Moreover, we found higher levels of sAA in older adults with T2D under an acute psychosocial stress, compared to older subjects with T2D in a control condition. Thus, our results support the idea that the TSST also provokes a greater SNS response in older T2D subjects. The result agrees with results found in healthy older people (Almela et al., 2011; Pulpulos et al., 2015), and confirms the relationship previously reported between sAA and perceived stress (Siddiqui et al., 2015). Although Strahler et al. (2010) found an attenuated sAA response to TSST in healthy older adults. These controversial results could be explained by the hypothesis that a decrease in sAA in older participants suggesting a lower stress reactivity with ageing (Kudielka et al., 2004b; Lavi et al., 2006; White et al., 1997). A second explanation would be the relative lack of studies on SNS markers in relation to acute stress in older population in general, and in T2D people in particular. Hence, further research in sAA response to acute stress in T2D people is clearly needed.

We failed to find stress effects on WM performance in our diabetic sample. Our findings agree with other studies in healthy older people (Pulopulos et al., 2015). This result supports the idea that older people may be less sensitive to the effects of stress on memory than young people (Hidalgo et al., 2019), as we previously reported for WM performance (Pulopulos et al., 2015). These results could be due to an age-related dysregulation of HPA-axis activity (Mizoguchi et al., 2009) and functional changes in the amygdala and hippocampus (Mather, 2006; Murty et al., 2009; St. Jaques et al., 2009) in older people.

In our study, WM retention rate was negatively related to the interaction between the sAA and cortisol responses to stress. This result suggests that, this type of memory would be affected by the interaction between both systems (HPA-axis and the SNS). It is important to take this into account in order to obtain more data in further investigations about the possible impact of stress on WM retention rate and how this might interfere in the T2D daily living and instrumental activities.

In summary, this study provides findings about the psychobiological response to acute psychosocial stress in older T2D patients. In addition, our findings add interesting information about the impact of acute stress in WM in older T2D people with adequate medical supervision, since this type of memory is crucial for autonomous self-care management and complex problem solving.

Study 2

Even though the impact of the exposure to an acute stressor on DM has been investigated in young (Cohen et al., 2020; Smeets et al., 2007), and older (dos Santos et al., 2017) healthy adults (for a review on this topic see Hidalgo et al., 2019), the studies with T2D samples are still lacking. Hence, the main aim of the second study of this thesis was to know the possible acute stress effects on declarative memory performance in T2D people. To do this, we compared the psychobiological response (i.e., affect, sAA, and C) to an acute psychosocial

stressor, the declarative memory performance and the impact of the acute stressor on declarative memory retrieval performance between 50 T2D and 50 healthy older participants (52 to 80 years old).

Results indicated lower positive affect and higher negative affect after the TSST in all participants, suggesting that the psychological response to TSST was similar between T2D and healthy older participants. This finding agrees with previous studies in both healthy (Hidalgo et al., 2020) and T2D (Vallejo et al., 2021) older people.

In addition, sAA response to TSST in T2D was also similar to healthy individuals. This result is in line with previous studies in healthy (Pulopulos et al., 2018) and in T2D (Vallejo et al., 2021) older people. Although, our result is in contrast to the attenuated sAA response reported by Strahler et al. (2010) in older healthy people. These mixed results could be due, firstly, to the fact that Strahler et al. (2010) used a shorter preparation phase (3 min), and secondly, included a younger sample (59-61 yrs) than our study (5 min; 52-80 years).

Regarding cortisol results, in contrast to the stronger cortisol response of T2D patients compared to healthy ones found by Buckert et al. (2022), we reported that T2D had similar cortisol response than healthy older people, since we did not find an effect of Group (T2D vs. healthy). Maybe, this lack of differences between groups could be due to all participants had similar motivation to perform the task, and/or because the T2D participants were medically treated. In addition, methodological differences such as the type of sample collected to measure cortisol levels (saliva vs. blood) could explain these mixed results. Together, more research is needed about psychobiological response to the TSST in T2D older people compared with healthy participants.

As we expected, the analyses in memory retrieval indicated that the information recall (memory retrieval for pictures, words and stories) was not affected by stress in T2D and healthy group. This result is in line with other previous studies that investigated DM in healthy older people (Pulopulos et al., 2013; Hidalgo et al., 2014, 2015). One explanation for these results could be the HPA axis dysregulation (Mizoguchi et al., 2009), changes in the SNS (Arnsten and Goldman-Rakic, 1985a, 1985b) and functional cerebral alterations (Mather, 2006;

Murty et al., 2009; St. Jaques et al., 2009) observed in older individuals. Moreover, this result supports the hypothesis that older people might be less sensitive to the psychosocial stress effects on memory than younger people (for a review see: Hidalgo et al., 2019).

In addition, we found that T2D participants recalled a smaller number of ideas (Rivermead test) in acquisition and retrieval session compared with healthy participants. Considering that DM is a cognitive domain dependent on hippocampal functioning, the hippocampus atrophy that diabetic people can suffer (Hayashi et al., 2011; Moran et al., 2013) could explain this result. It is important further studies linking acute stress and DM in T2D people considering the hippocampus atrophy in order to confirm this hypothesis.

Finally, after TSST, only healthy participants with higher cortisol concentrations, recalled, marginally, less words. This finding is in line with studies with young people (Hidalgo et al., 2015; Smeets, 2011). However, our result is in contrast to those reported by Pulpulos et al. (2013) who failed to find an association between stress and memory retrieval. One explanation for this result could be the difference formula used for calculate de cortisol reactivity. We calculated the cortisol reactivity subtracting the basal levels from the maximum levels after the onset of the stressor, while Pulpulos et al. (2013) calculated this index according to the trapezoid formula (Pruessner et al., 2003). Future research may help to clarify this issue.

Together, our results obtained in this second study point out that T2D patients show a similar psychobiological response to an acute psychosocial stressor than healthy ones, and extend in a sample of older T2D patients, the previous idea suggesting older people are less sensitive to stress effects on memory retrieval. In addition, provides important information about difficulties in declarative memory in T2D patients.

Study 3

The third study of this thesis investigated: i) if the diurnal cortisol cycle was different between healthy and T2D older people, ii) if T2D people had a worst cognitive performance compared with healthy individuals, iii) the possible

relationships between two indexes of the diurnal cortisol cycle (i.e., CAR and DCS), and cognitive performance (DM, WM, and EF) in T2D people in comparison to healthy individuals, iv) the role of depression in the relationships among cortisol indexes and cognitive functions. In this last study, the sample consisted of 51 DT2 patients and 51 healthy participants. To measure the diurnal cortisol cycle, participants provided 4 saliva samples during 2 consecutive working days, and cognitive performance was evaluated by different memory and executive function tests.

Our results point out no differences between CAR and DCS among T2D and healthy people. The literature provides different results regarding this. About the CAR, no differences were observed in other groups (Hackett et al., 2014; Johar et al., 2016), but flatter CAR (Bruehl et al., 2009; Panagiotou et al. (2021) and lower CAR in T2D patients have also been reported (Lederbogen et al., 2011). Regarding DCS, our results are in line with Bruehl et al. (2009), although Hackett et al. (2014) observed a flattened DCS in T2D. These differences could be explained, at least in part, in the different way each index is calculated in the different studies.

We also provide data on different cognitive domains in T2D compared to healthy people. Regarding DM, differences in both tests used were observed. Thus, after RAVLT administration, worse performance was evidenced on the total learning and the immediate recall, but not in delayed recall, in the T2D group in comparison to healthy group. This finding agrees with the meta-analyses of Palta et al. (2014) and Sadanand et al. (2016) who indicated more T2D associated deficits reported in the immediate than in the delayed recall. After the RBMT administration, a significant worse performance in T2D patients but not differences depending on time, were observed, in comparison to healthy people. The differences in the results provided by the two tests could be due to several circumstances. The material to be recalled. RAVLT is a list of unstructured verbal memory, therefore requires a higher cognitive demand for learning because of the lack of semantic context. In contrast, RBMD provides material in paragraphs with a semantic context (Palta et al., 2014), which can help to a better recall. Despite the differences between the two tests, our results provide evidence that

T2D patients may have greater difficulty remembering certain aspects of verbal material involving prompts or conversations that may be important for proper monitoring of the disease.

In relation to WM, T2D presented worse performance on DS-Forward index, LNS, and marginal differences in DS-Backward. Our results are in line with Mollon et al. (2020) when compared T2D with healthy people. In contrast, Mallorquí-Ballé et al. (2018) found no differences between T2D and controls in DS subtests. These mixed results among WM tests could be explained because of the complexity of the execution test. For example, LNS test suppose more demands than DS-Backward, since implies manipulation of more information at very short time period. Furthermore, it should be noted that WM is one of the cognitive domains where the greatest differences in midlife are observed (Pelimanni and Jehkonen, 2018).

Regarding the EF, T2D participants had worse performance in TMT in comparison to healthy people, confirming previously results (Palta et al., 2014; Mansur et al., 2018), but no differences between groups were found in the Stroop interference test.

We also explored the relationship between cortisol indexes and cognitive performance. We only find a significant negative relationship between CAR and Stroop interference in the healthy group. In this sense, there is evidence that a larger CAR is associated with improvements in cognition at older ages (Gardner et al., 2019). Contrary to our expectations, no associations between DCS and DM were showed.

Considering the association between T2D, depressive symptomatology and cognitive function, we explored if depression could affect the relationships studied. We found that higher CAR was related to lower Stroop interference at middle levels of depression, while higher DCS was related to higher scores in RAVLT delayed recall at lower levels of depression. This suggests that people, regardless T2D and healthy group, with low to moderate levels of depressive symptomatology and higher levels of CAR and steeper DCS may have better cognitive flexibility, resistance to interference and degree of cognitive complexity, as well as long term memory retention. It has been speculated a

biological association between depression, stress and T2D which could be due to a HPA axis dysregulation and hyperactivation (Gragnoli, 2012). No previous studies have conducted moderation analyses between cortisol indexes and cognitive performance attending for depression. It is important to delve on these results considering the high prevalence of T2D, cognitive dysfunction and depression comorbidity.

In sum, this third study provide evidence about worse cognitive performance, specifically some outcomes of DM, WM and EF in T2D patients. These cognitive difficulties may increase the risk of an impact on the self-care of these patients. In addition, this study provides new insights into the associations between diurnal cortisol profile, cognitive function and depression, as assessed by a comprehensive testing protocol, in a T2D group compared to healthy participants. It is important to note that cognitive dysfunction is a big risk of inadequate diabetes management from inadequate glycemic care. These results could serve as a basis for interventions to improve diabetic care. More research is needed in this area, considering the incidence of T2D, its relationship with depression and the increasing ageing of the world's population.

Limitations and strenghts

Each study included in this thesis contains specific limitations. In this section some general aspects that should be considered to interpret the results will be adressed. Firstly, we were conservative and selected a homogeneous T2D sample (first study) and matched healthy controls (second and third studies), in terms of sex, age, and other sociodemographic characteristics, in order to avoid as many confounding factors as possible. For this reason, we have a limited number of participants and, as a consequence, we could not study possible gender differences with possible limitations in the ability to detect some effects and generalize the results. In view of this, future studies should investigate the role of gender in this population, including bigger sample sizes. In addition, in the first study we included a control group with T2D patients who were not

subjected to the stress condition, but we did not include a control group of healthy individuals.

Therefore, future studies should add healthy older individuals to compare with T2D, on the relationship between the HPA-axis and WM performance. In the second and third studies, the order of the memory tasks administration was not counterbalanced. Further studies should counterbalance the order of the memory tests in order to confirm the lack of both stress effects on memory retrieval (second study) and relationship between cortisol indexes of HPA-axis basal functioning and WM, DM and EF performance.

Despite the limitations, the present doctoral thesis has some strengths. The first and second studies provide interesting findings about the psychobiological response to acute psychosocial stress in T2D patients with adequate medical supervision. Interestingly, the first study is, to the best of our knowledge, to investigate the sAA response in this clinical population.

In addition, the second study supposes the first attempt to study empirically the impact of acute stress on declarative memory in diabetic patients, extending the understanding of the stress-memory link to other unhealthy populations.

The third study provides evidence, for the first time, about the relationship between HPA-axis basal functioning and three cognitive domains (i.e., DM, WM and EF) assessed with a broad number of cognitive tests. Moreover, to measure the diurnal cortisol cycle, saliva samples were collected in two weekdays, as has been clearly recommended.

Finally, the samples of the three studies are defined population without any concomitant disease that could be related to cognitive deficits.

Future directions

The results obtained in this dissertation raise new important questions that should be explored in future research. It would be important to include more participants for studying possible gender differences in DM, WM and EF performance in relation to C and sAA levels. In addition, a larger sample of participants could strengthen the current results on mood and physiological responses to an acute psychosocial stressor in this population as well it would permit also to separate age groups and improve the knowledge about the specific characteristics and speed of the ageing process in T2D people. This thesis included cross-sectional studies investigating the acute stress response and its impact on cognitive function, however, there is a need for longitudinal studies encompassing chronic stress and its relationship with cognitive domains over the years in older T2D to shed light on disease progression, and to establish temporal correlations between disease and cognitive impairment. Longitudinal studies are complex but necessary to study adequately the different trajectories of this disease in relation to stress, depression and dementia to prevent and control their evolution.

Different studies have administered different psychological tests, for that reason, there is a need to develop a standardised neuropsychological protocol for assessing cognitive impairment in T2D. Future studies may contemplate an extensive and careful neuropsychological protocol testing that could benefit in the understanding of possible difficulties in the organization, and daily planning that living with this disease entails for this population.

Further studies should also be conducted to screen for DM and EF impairments to help older T2D patients who present problems in their daily autonomy to gain adherence to medical treatment, dietary control, and functional independence. Finally, depression symptomatology seems to be an important factor to consider in the link between HPA-axis basal functioning and cognitive function. So, it should be considered to improve possible difficulties in self-care and reduce the problems associated with the disease.

Chapter 7

Main Conclusions

The following general conclusions can be drawn from the studies including in this dissertation:

1. The TSST is capable to produce the psychobiological response previously reported in healthy people (mood, sAA and cortisol response), in medically treated patients with TD2.
2. The exposure to the TSST did not affect WM performance in T2D patients, as previously described in healthy people.
3. WM performance is related to the interaction between the HPA axis and the SNS.
4. Stress did not affect the memory retrieval for pictures, words, or stories in T2D and healthy older people.
5. Cortisol response to acute stressor is negatively related to retrieval of words, but not of pictures and stories only in healthy older adults and not in T2D older people.
6. T2D participants recalled a smaller number of ideas about stories in acquisition and retrieval session in comparison to healthy participants, but not group differences were found in word nor pictures.
7. Older adults with T2D reported worse cognitive performance (i.e., WM, DM and EF) compared to healthy individuals.
8. T2D participants showed similar diurnal cortisol cycle than healthy older people
9. Diurnal cortisol pattern, as reflex of HPA-axis basal functioning, did not appear significantly related to the impaired cognitive performance observed in T2D participants.
10. The relationship between higher CAR and better cognitive flexibility (Stroop interference) was confirmed in the healthy but no in the T2D participants.
11. When depression is considered, a negative significant CAR - Stroop interference relationship for low and middle levels of depression appeared
12. Finally, a significant positive relationship between DCS and RAVLT delayed recall for low depression was confirmed.

Chapter 8

Resumen general en español

Introducción general

Diabetes tipo II: definición, prevalencia e impacto en la cognición

La diabetes tipo II (DT2) se considera el trastorno metabólico crónico de mayor prevalencia, asociado a la resistencia a la insulina y la hiperinsulinemia (Chatterjee et al., 2017; Khalid et al., 2022; Umegaki, 2014). Esta enfermedad se debe al daño de la célula β pancreática, a la deficiencia de secreción de insulina o a la no utilización de insulina, lo que provoca niveles más elevados de glucosa en sangre (Padhi et al., 2020).

La diabetes afecta a más de 500 millones de personas en todo el mundo y aumentará a más de 1.300 millones en 2050, según la Carga Mundial de Morbilidad (GBD, 2021). La Federación Internacional de Diabetes (FID, 2022) ha destacado que la DT2 es una de las emergencias sanitarias mundiales de más rápido crecimiento del siglo XXI. Más del 90% de todos los casos de diabetes en el mundo se deben a la DT2 (Ali et al., 2022).

Las causas de la DT2 no se conocen por completo, pero se ha reconocido un fuerte vínculo entre el estilo de vida y su contribución al desarrollo de la DT2, en particular factores relacionados con el sedentarismo, la inactividad física, el sobrepeso o la obesidad (Dedoussis et al., 2007; Kahn et al., 2014). Además, los antecedentes familiares de DT2 implican un mayor riesgo de desarrollar la enfermedad. En esta línea, algunas revisiones han subrayado la importancia de los factores genéticos en el desarrollo de DT2 (Laakso, 2019; Lin et al., 2017). Se han encontrado más de 400 variantes genéticas en personas con DT2, con un efecto pequeño o moderado en el riesgo de padecer la enfermedad (Mahajan et al., 2018). Por lo tanto, el conocimiento de la historia clínica del paciente será importante para proporcionar información a las personas con antecedentes de DT2. Además de todas las causas mencionadas, la edad también es un factor determinante frente a la aparición de la DT2. Por tanto, el creciente envejecimiento de la población en todo el mundo (FID, 2022), junto con la reducción de la actividad física y la exposición a factores cardiometabólicos, ha

provocado un aumento de la DT2 en la población de mayor edad (Khan et al., 2020). En esta línea, la DT2 se convierte en una enfermedad común en las personas de 50 años o más (Bommer et al., 2017), observándose la mayor prevalencia de DT2 en las personas de 75-79 años (FID, 2022). Además, la mayor prevalencia de multimorbilidad alcanza el 40% en los adultos mayores con DT2 (Piette, 2006; Wolff et al., 2002). También hay pruebas de afecciones asociadas a la edad que disminuyen la calidad de vida de los adultos con DT2, como el dolor crónico, la incontinencia y las caídas (Laiteerapong et al., 2011).

Con el tiempo, el desequilibrio metabólico crónico en la DT2 se asocia a múltiples complicaciones como retinopatía, cetoacidosis, nefropatía, infarto de miocardio, ictus y amputación de miembros inferiores (Harding et al., 2019; FID, 2015). El tratamiento de estas complicaciones supone una carga económica cada vez mayor para los sistemas sanitarios (Zhang et al., 2010), ya que los pacientes con DT2 requieren un mayor uso de medicación y tienen más probabilidades de necesitar cuidados a largo plazo (Unwin et al., 2010).

Un punto muy importante es la relación de la DT2 con la salud mental, concretamente con la depresión (Farooqi et al., 2022; Lindekilde et al., 2022; Van Sloten et al., 2020). El mayor riesgo de depresión puede deberse a las complicaciones relacionadas con la diabetes (Chireh et al., 2019), a la carga psicológica tras el diagnóstico, al control de la hiperglucemia o al tratamiento de la propia enfermedad (Anderson et al., 2001; Engum, 2007). Teniendo en cuenta la alta prevalencia de DT2 y depresión, el cribado regular de la misma es de gran importancia, siendo esto recomendado por las guías clínicas para el manejo de la diabetes (FID, 2005; NICE, 2009).

Asimismo, la literatura reciente ha mostrado un mayor riesgo de trastornos de ansiedad en pacientes con DT2 (Benmaamar et al., 2022; Mersha et al., 2022). En esta línea, se reportó un 56% mayor prevalencia de síntomas ansiosos en aquellos pacientes que conocían el diagnóstico de DT2 (Arteaga-Zarate et al., 2022; Meurs et al., 2016). En estos pacientes, los niveles elevados de ansiedad han demostrado estar asociados con un empeoramiento de los

niveles de glucosa en sangre, un aumento del índice de masa corporal (IMC) (Balhara y Sagar, 2011) y una reducción de la calidad de vida (Penckofer et al., 2012). Por todo lo anterior, es recomendable realizar un seguimiento psicológico a los pacientes diabéticos tanto en el momento del diagnóstico como en las citas posteriores.

Además de todas estas complicaciones, la literatura ha reconocido cada vez más la relación entre la DT2 y un mayor riesgo de disfunción cognitiva (Casagrande et al., 2021; Dao et al., 2022; Umegaki et al., 2013). En esta línea, un estudio longitudinal de población mostró una asociación entre la DT2 en ancianos y el deterioro cognitivo acelerado (Hassing et al., 2004). Se ha observado que el riesgo de deterioro cognitivo acelerado es entre 1,5 y 2,0 veces mayor en pacientes con DT2 que en participantes sanos (Biessels y Despa, 2018; Xiong et al., 2020).

Las dificultades cognitivas asociadas pueden tener efectos negativos en la atención y la gestión de la enfermedad (Tomlin y Sinclair, 2016), dando lugar a complicaciones como la hipoglucemia (Lee et al., 2017). Sin embargo, la DT2 suele presentarse con otras comorbilidades también relacionadas con el deterioro cognitivo, lo que dificulta establecer una relación causal entre estos procesos (Li et al., 2019).

Varios mecanismos podrían contribuir a explicar la relación entre la DT2 y el deterioro cognitivo. Así, la combinación de daño vascular y neurodegenerativo puede ser la causa de la disfunción cognitiva en pacientes con DT2 (Cholerton et al., 2016), aunque el impacto de la DT2 en la cognición no se ha determinado claramente (Sandhir y Gupta, 2015).

Entre algunas de las hipótesis que se barajan, se ha destacado la vulnerabilidad del cerebro a los efectos adversos de la DT2 (Carvalho et al., 2020). El cerebro es rico en receptores de insulina en áreas relacionadas con la memoria, como el hipocampo (Pomytkin et al., 2018), un área cerebral que desempeña un papel en el aprendizaje y la memoria (Lee et al., 2012). Se ha

demostrado que una menor sensibilidad cerebral a la insulina puede provocar un fallo energético, lo que conduce a un deterioro de la plasticidad cerebral, el crecimiento nervioso y la función cognitiva (Cai et al., 2017; Ng et al., 2016).

Se ha propuesto otro mecanismo hipotético subyacente al vínculo de alteración cognitiva de la DT2 a través de la sensibilidad a la insulina mediada por la hiperglucemia en el cerebro, que afectaría a la plasticidad sináptica en el hipocampo. Esto podría comprometer la memoria, conduciendo potencialmente a una disfunción cognitiva (Kerti et al., 2013; Spinelli et al., 2019). En esta línea, la concentración excesiva de glucosa en la DT2 tiene efectos tóxicos sobre las neuronas cerebrales. El mantenimiento de niveles elevados de glucosa conduciría a la formación de productos finales de glicación avanzada (AGE), provocando efectos de alta toxicidad sobre las neuronas (Yamagishi et al., 2007).

Las anomalías microvasculares también afectan al cerebro en pacientes con DT2 (Van Sloten et al., 2020), debido a la hipertensión que podría causar rigidez arterial (Climie et al., 2019). Esto podría explicar por qué el cerebro se ve afectado con mayor frecuencia por enfermedades microvasculares en la DT2 (Stehouwer, 2018), contribuyendo a un mayor riesgo de disfunción cognitiva en pacientes con esta enfermedad (Feinkohl et al., 2019; Reijmer et al., 2013; Van den Berg et al., 2009).

Para realizar las tareas de autocuidado en la DT2, es importante que las habilidades cognitivas permanezcan preservadas (Tomlin y Sinclair, 2016). Sin embargo, la literatura ha demostrado deterioro en múltiples dominios cognitivos en pacientes con DT2 (Abbatecola et al., 2010; Dyer et al., 2020; Strachan et al., 2009), presentándose deterioro en atención (Cukierman-Yaffe et al., 2009; Messier et al., 2010), memoria declarativa (MD) (Geijselaers et al., 2015; Li et al., 2021; Ravona-Springer et al., 2014), memoria de trabajo (MT) (Backeström et al., 2021; Dyer et al., 2020; Monette et al., 2014; Palta et al., 2014) y función ejecutiva (FE) (Biessels y Despa, 2018; Casagrande et al., 2021; Cui et al., 2014; Moheet et al., 2015; Munshi et al., 2006; Sadanand et al., 2016), especialmente

en aquellos de mayor edad y que padecen DT2 durante más tiempo (Velayudhan et al., 2010).

La atención se refiere al estado de observación y alerta que nos permite ser conscientes de lo que ocurre en nuestro entorno (Ballesteros, 2002). Se han demostrado dificultades atencionales en pacientes con DT2 (Manschot et al., 2006; Moheet et al., 2015). En esta línea, Ruis et al. (2009) encontraron que los DT2 de mayor edad presentaban un rendimiento atencional significativamente peor. Además, en un metaanálisis (Pelimanni y Jehkonen, 2018) y en revisiones sistemáticas (Xue et al., 2019; Wang et al., 2021), también se evidenció un peor rendimiento en pacientes con DT2. Una atención adecuada permite ignorar información o detectar cambios en el entorno que ponen en peligro la vida. Por lo tanto, la atención se considera un dominio cognitivo de gran importancia (Katsuki y Constantinidis, 2014).

Con respecto a la MD, se han reportado alteraciones en la MD verbal en pacientes con DT2 (Yau et al., 2014). La MD es un importante sistema de aprendizaje (Ullman y Pullman, 2015) que se refiere a la adquisición y recuperación de episodios, hechos y eventos que permiten contrastar y comparar el material evocado (Squire, 2004). La literatura ha informado de un menor rendimiento en el recuerdo verbal inmediato y diferido (Debling et al., 2006; Grodstein et al., 2001; Yau et al., 2014). Sin embargo, en un estudio longitudinal más reciente, el peor rendimiento en la memoria a corto y largo plazo no fue estadísticamente significativo tras el ajuste por factores de riesgo vascular (Xie et al., 2022).

En cuanto a la MT, se ha demostrado un deterioro en las personas con DT2 en comparación con no DT2 en estudios transversales (Backeström et al., 2021; Zhang et al., 2016), longitudinales (Xie et al., 2022) y de revisión (Kim, 2019). La MT es un proceso cognitivo de manipulación y almacenamiento temporal de información en el cerebro, siendo importante para la realización de muchas tareas complejas (Baddeley et al., 1996, 2003). Algunos estudios han demostrado que la MT estaba alterada en pacientes con DT2 sin complicaciones,

en comparación con los controles sanos (Cansino et al., 2021; Zhang et al., 2016). Además, Mythri et al. (2017) también demostraron que las puntuaciones de la MT disminuían en los pacientes con DT2. Teniendo en cuenta los estudios mencionados anteriormente, la dificultad en este ámbito es evidente.

Las FE son el conjunto de procesos de control responsables de la planificación, el razonamiento, la coordinación, la secuenciación de la resolución de problemas y la monitorización de las operaciones cognitivas (Hull et al., 2008; Qiu et al., 2006). Múltiples estudios han evidenciado deficiencias en una variedad de tareas ejecutivas en adultos mayores con DT2 (Van den Berg et al., 2009; Yeung et al., 2009) y un mayor riesgo de problemas ejecutivos. Por ejemplo, Yeung et al. (2009) evaluaron el control inhibitorio mediante la subprueba Stroop (parte III). Incluyeron el Color Trails 2 para evaluar el mismo dominio, pero sin la influencia del lenguaje. Los participantes con DT2 obtuvieron peores resultados que los controles sanos en la prueba Color Trails 2, aunque no se observaron diferencias en las pruebas Stroop. En otro estudio, Fitas et al. (2010) mostraron peores resultados en individuos con DT2 en comparación con los no diabéticos.

Además, estudios longitudinales mostraron una evolución desfavorable del rendimiento cognitivo a lo largo de los años, en pacientes mayores con DT2, en comparación con personas sanas (Fontbonne et al., 2001). Asimismo, los pacientes con DT2 presentaban un mayor deterioro del rendimiento en las actividades instrumentales de la vida diaria, en comparación con las personas sin DT2 (Kuo et al., 2005). En resumen, el síndrome disejecutivo puede presentarse en personas mayores con DT2, aumentando el riesgo de repercutir en su autocuidado.

Teniendo todo esto en cuenta, se hace necesario estudiar cuáles son los mecanismos neurológicos en la DT2 que podrían conducir a un peor rendimiento cognitivo en esta población.

El concepto de estrés

El estrés se define como una respuesta biológica resultante de estímulos extrínsecos o intrínsecos (Yaribeygi et al., 2017). La respuesta de estrés puede entenderse a nivel físico, emocional o cognitivo/conductual, pudiendo acarrear graves consecuencias para el organismo si se mantiene a largo plazo (Bao et al., 2008).

El concepto actual de estrés se ha desarrollado a partir de las aportaciones de Cannon y Selye. En 1929, Cannon utilizó por primera vez el término "homeostasis" para describir la tendencia a mantener la estabilidad fisiológica en un equilibrio dinámico. Este autor indicó que una variedad de amenazas a la homeostasis provocaba una respuesta simpato-adrenal subyacente a la "lucha o huida" (Cannon, 1929). Selye descubrió que la activación del eje HPA parecía ser una respuesta común a una variedad de "agentes nocivos", que inician una reacción en el cuerpo llamada "síndrome general de adaptación" (GAS) (Selye, 1936; 1974). El GAS se caracteriza por tres fases por las que pasa el cuerpo cuando se experimenta estrés: i) Fase de alarma, cuando el cuerpo se prepara para la "lucha o huida"; ii) Fase de resistencia en la que se produce la resistencia al estrés; y iii) Fase de agotamiento, cuando el estrés se mantiene por largos periodos de tiempo, el cuerpo entra en una etapa de agotamiento, que puede tener un efecto peligroso para el organismo (Selye, 1936). Así, el estrés era considerado una respuesta indeterminada del organismo a cualquier demanda que se le plantea (Selye, 1956; 1974).

Sin embargo, en los últimos 50 años, la neurociencia ha ampliado las ideas sobre el estrés a lo largo de muchas dimensiones (McEwen y Akil, 2020). La respuesta biológica al estrés se ha manifestado no solo en términos de alcanzar un estado negativo, sino como un conjunto de mecanismos acumulativos, continuos y adaptativos de evaluación ambiental positiva que permiten al individuo anticipar y afrontar los desafíos futuros con el fin de mejorar la supervivencia a lo largo de la vida (McEwen y Akil, 2020). Además, se ha demostrado que un nivel inadecuado o excesivo de estrés puede perjudicar la

composición corporal, el crecimiento y el desarrollo, así como conducir a estados patológicos. Esto confirma que el estrés incontrolable es negativo y puede ser perjudicial física y psicológicamente (McEwen y Akil, 2020). Por el contrario, un nivel óptimo de estrés parece ser adecuado para la salud (Chrousos, 2009).

El concepto de homeostasis se ha utilizado con el significado primario de mantener la estabilidad interna a través de la constancia. En 1988, Sterling y Eyer utilizaron el término "alostasis" definido como el proceso de mantener y adaptar activamente la estabilidad del organismo a través de la acción de varios mediadores, como el cortisol. Más tarde, para describir el proceso de estrés, el término "carga alostática" fue utilizado por primera vez por McEwen (McEwen, 2003; 2007). Se define como el costo energético al que se ve sometido el organismo al adaptarse a situaciones físicas o sociales adversas con el fin de alcanzar un punto de equilibrio (McEwen, 2000).

Teniendo en cuenta el impacto que tiene el estrés sobre la salud del individuo, es importante señalar que existe cierta evidencia de una asociación entre el estrés y DT2. En esta línea, el estrés crónico parece jugar un papel importante en el origen (Surwit et al., 1992) y desarrollo de la DT2 (Eriksson et al., 2013; Hackett y Steptoe, 2016; Heraclides et al., 2009). El estrés crónico puede influir en la aparición de la enfermedad al aumentar los problemas metabólicos, produciendo hiperglucemia que puede conducir a resistencia a la insulina (Marcovecchio y Chiarelli, 2012; Sharma et al., 2022). Además, Mooy et al. (2000) concluyeron que los acontecimientos vitales significativos podrían conducir a la aparición de la DT2 en individuos con predisposición a la enfermedad, siendo esto independiente del ejercicio físico o el nivel educativo.

En la siguiente sección se proporcionan información sobre los mecanismos subyacentes a la respuesta al estrés y su relación con la DT2.

Respuesta al estrés

La respuesta fisiológica al estrés tiene dos componentes básicos: una respuesta rápida, mediada por el sistema simpático-adrenomedular (SAM), y una respuesta más lenta mediada por el eje HPA (Chu et al., 2022). En respuesta a situaciones de estrés, la activación del sistema SAM y del eje HPA, como mecanismos adaptativos, permiten al cuerpo humano mantener la estabilidad fisiológica. Una falta de regulación de estos sistemas puede conducir a anomalías metabólicas (Calandra-Buonaura et al., 2015).

Ante una amenaza, se produce la respuesta de lucha o huida, iniciada por el SAM, en conexión con el eje HPA (Leonard y Myint, 2009). Por un lado, la activación del SAM conduce a un aumento de la secreción de epinefrina (E) y norepinefrina (NE), que se inicia en la médula y se extiende a la circulación, elevando los niveles de NE en el cerebro. Los receptores β -adrenérgicos y α -adrenérgicos del sistema nervioso central son aquellos con los que interactúan la E y la NE liberadas. Una vez liberadas, la E y la noradrenalina (NA) se unen a receptores específicos unidos a la membrana para iniciar una vía de señalización intracelular de AMPc. La activación de estos receptores provoca la contracción de las células cardíacas, lo que lleva a un aumento de la frecuencia cardíaca, la presión arterial y los niveles de glucosa, el aumento del consumo de oxígeno, así como la activación conductual (aumento del estado de alerta, vigilancia y cognición). Por otro lado, cuando se activa el eje HPA, se produce la secreción de la hormona liberadora de corticotropina (CRH) en el hipotálamo, seguida de un aumento de la producción y la secreción de la hormona adrenocorticotropina (ACTH) de las células pituitarias. Esto conduce a la síntesis y secreción de glucocorticoides (GC) a través de la corteza suprarrenal. Los GC más tarde forman parte de un ciclo de retroalimentación negativa del eje HPA: la unión de los GC a los receptores de glucocorticoides (GR) en el PVN y la pituitaria inhibe la actividad del eje HPA a través de la regulación negativa de la ACTH y su síntesis y liberación.

Los GC se unen a los receptores de glucocorticoides (GR) y de mineralocorticoides (MR). Los GC influyen en los mecanismos adaptativos y las respuestas conductuales al estrés (Pfau y Russo, 2015; Russo et al., 2012), alterando la remodelación estructural, la plasticidad sináptica y los perfiles de expresión génica. Además, los GC modulan la función micro glial, manteniendo un estado proinflamatorio (Frank et al., 2013; 2015). En la periferia, los GC inducen apoptosis en linfocitos T, monocitos y macrófagos. Además, protegen a las células inmunes en el tejido inflamado al reducir la respuesta inflamatoria (Amsterdam et al., 2002).

Así, el eje HPA se considera un eje neuroendocrino complejo encargado de generar la respuesta del organismo a un estresor emocional o físico que pueda comprometer el equilibrio homeostático del organismo (Habib et al., 2001). Estas variaciones a nivel endocrino previenen posibles consecuencias negativas para el individuo (Leonard y Myint, 2009).

Las ramas simpática y parasimpática son los componentes del sistema nervioso autónomo (SNA) que regulan las funciones viscerales (Sanvictores et al., 2023). El SNA, el eje HPA y el sistema inmunitario trabajan juntos para lograr la respuesta hormonal e inflamatoria al estrés (McEwen et al., 2015; Ulrich-Lai y Herman, 2009). Así, un estresor agudo y la respuesta consecuente pueden dar lugar a un mecanismo adaptativo del sistema endocrino con efectos protectores. Por el contrario, el estrés sostenido y repetitivo puede producir cambios perjudiciales, dando lugar a alteraciones a largo plazo del eje HPA y a condiciones patológicas (De Kloet et al., 2005).

La CRH coordina las respuestas autónomas, cognitivas y conductuales a los estímulos de estrés, complementando las respuestas endocrinas del eje HPA (De Kloet et al., 2005). La CRH produce modificaciones en el sistema simpático suprarrenal y el eje HPA, incluida la activación de macrófagos centrales y periféricos y la producción de agentes proinflamatorios, así como una acumulación de grasa abdominal e hipertensión. El aumento de la producción de CRH en el hipotálamo puede mantener la activación prolongada del eje HPA. La CRH también ejerce un efecto activador en el sistema simpático central al activar el locus coeruleus. La respuesta al estrés agudo está regulada por un circuito de

retroalimentación, que ralentiza la liberación de CRH y ACTH (Leonard y Myint, 2009).

En una situación de estrés crónico, la resistencia a los GC se asocia a una disminución de los procesos antiinflamatorios debido a un estado mantenido de mediadores proinflamatorios (Cohen et al., 2012). En la respuesta al estrés, una activación crónica de los sistemas biológicos puede conducir a una reactividad fisiológica desregulada que contribuya a resultados negativos para la salud con una mayor vulnerabilidad a algunos trastornos (McEwen y Wingfield, 2003) como los síndromes metabólicos, la resistencia a la insulina y la DT2 (Black, 2006). En la DT2, se ha demostrado que la biología relacionada con el estrés está alterada y pueden estar presentes múltiples componentes biológicos como el aumento de la inflamación, el cortisol y la desregulación del sistema cardiovascular, lo que refleja una carga alostática crónica (Hackett et al., 2017).

En resumen, las situaciones de estrés y su respuesta desadaptativa se han asociado con la DT2. Por lo tanto, sería importante medir los niveles de estrés en estos pacientes, brindando información sobre qué biomarcadores específicos de la respuesta de estrés se asocian a la DT2. Este contenido se abordará en la siguiente sección.

Respuesta al estrés en la DT2

Las muestras de saliva se han utilizado para estudiar las respuestas de alfa-amilasa salival (Aas) y cortisol debido a sus ventajas en comparación con otras técnicas (Prasad et al. 2016). Por ejemplo, en comparación con la sangre, una de las ventajas más importantes es que la saliva es una muestra mínimamente invasiva (Tvarijonaviciute et al., 2020). Además, en comparación con otras muestras no invasivas como la orina y las heces, la saliva es más conveniente para muestrear debido a la simplicidad para el almacenamiento y el transporte (Yoshizawa et al. 2013).

En cuanto a la respuesta de Aas en personas mayores con DT2, la investigación es escasa. Solo Siddiqui et al. (2015) utilizaron el biomarcador de Aas en participantes con DT2, informando una relación positiva entre el estrés

percibido y los niveles de Aas. Se necesitan más estudios para profundizar en la asociación entre los niveles de AAS y la DT2.

En relación con la respuesta del eje HPA, se ha evidenciado un aumento de la respuesta del eje HPA en individuos con DT2 en estudios de estimulación neuroendocrina. En esta línea, Bruehl et al. (2007) demostraron un aumento en la respuesta de CRH. Estos resultados concluyeron una anormalidad en la sensibilidad de retroalimentación del eje HPA, presentando tales resultados como un posible predictor de DT2 (Taheri et al., 2018). Reynolds et al. (2005; 2010) también informaron niveles elevados de cortisol matutino y vespertino en personas mayores con DT2, y niveles elevados de ACTH y cortisol después de estrés repetido (Reynolds et al., 2001).

Con respecto al estrés agudo, algunos estudios mostraron descensos en el cortisol inmediatamente después de un estresor psicológico, analizándolo como una disrupción en los sistemas biológicos relacionados con el estrés (Carvalho et al., 2015; Steptoe et al., 2014). Faulenbach et al. (2011) observaron niveles más altos de cortisol en participantes con DT2 después de la exposición a un estresor psicosocial agudo (Trier Social Stress Test, TSST). Esta prueba también fue utilizada por Monzer et al. (2022), quienes demostraron un deterioro del funcionamiento del SNA, manifestado por una recuperación más lenta de la frecuencia cardíaca y un aumento atenuado de la misma en respuesta al estrés psicosocial. En este contexto, parece existir una clara relación entre la DT2 y el estrés agudo. Por ello, este tema debe considerarse como una posible causa a tener en cuenta en el manejo de la diabetes. Dada la escasa investigación sobre este tema en la población con DT2, son necesarios más estudios.

Debido a los pocos estudios publicados que evalúan la respuesta psicobiológica a un estresor agudo y su efecto sobre el rendimiento cognitivo, el capítulo tres intenta abordar esta cuestión. Además, en el capítulo cuatro, se explora el efecto de un estresor agudo sobre la MD, MT y FE. En vista de la necesidad de investigar la relación entre la respuesta basal del eje HPA con el rendimiento cognitivo en múltiples dominios, el capítulo cinco se centra en esta relación.

El patrón diurno del cortisol en la DT2

Como se ha mencionado, el cortisol salival refleja los cambios relacionados con el estrés en el eje HPA, tanto en respuesta a un factor estresante como en condiciones basales. En relación con esto último, el ciclo diurno del cortisol se considera un indicador de la salud del eje HPA, ya que refleja el funcionamiento del eje HPA en situaciones normales o sin estrés y la exposición al estrés crónico está relacionada con la desregulación del eje HPA (Miller et al., 2007).

Es bien sabido que el ritmo circadiano del cortisol muestra un patrón caracterizado por un rápido aumento de los niveles de cortisol al despertar (que alcanza su punto máximo entre los 30 y los 45 minutos) y una disminución gradual a lo largo del día (Pruessner et al., 1997). Se puede construir una curva de cortisol diurna mediante la recolección de saliva cronometrada y puede incluir los siguientes índices: (i) respuesta de cortisol al despertar (CAR; el aumento de cortisol que ocurre entre los 30 y los 45 minutos después de despertarse); y (ii) la pendiente diurna de cortisol (DCS; la disminución de cortisol durante el día, calculada frecuentemente como la diferencia entre el cortisol al despertar y el cortisol vespertino (Adam et al., 2017).

En estudios que evalúan el CAR en la DT2, se ha encontrado un cortisol salival diurno aplanado, en comparación con individuos sanos (Lederbogen et al., 2010; Panagiotou et al., 2021). Aunque en otros estudios no se han observado diferencias (Carvalho et al., 2015; Hackett et al., 2014). En cuanto a la pendiente diurna de cortisol (DCS), Vreeburg et al. (2009) no observaron asociación entre la DT2 y la DCS. Sin embargo, estudios posteriores mostraron una pendiente aplanada en pacientes con DT2 (Hackett et al., 2014; Lederbogen et al., 2011). Por último, con respecto a los niveles de cortisol a la hora de acostarse, se ha encontrado un nivel elevado de cortisol salival antes de acostarse en personas mayores con DT2 (Hackett et al., 2014; Johar et al., 2016; Lederbogen et al., 2011), en comparación con las personas sanas.

Estrés y función cognitiva en la DT2

Estrés agudo y rendimiento de la memoria en la DT2

La DT2 se asocia a situaciones de alto estrés como consecuencia de las demandas y el control al que se ve sometida una persona con una enfermedad crónica (Skinner et al., 2019). Los episodios de estrés agudo afectan al organismo (Juárez, 2020) a través de cambios hormonales que provocan una desregulación corporal (Hoogendijk et al. 2019). Esto puede afectar al control glucémico en pacientes con DT2 (Marcovecchio y Chiarelli, 2012). En esta línea, episodios de hipoglucemia (Aung et al., 2012) e hiperglucemia (Cerasuolo e Izzo, 2017) se han asociado a alteraciones de la MT en la población con DT2 (Ryan et al. 2006).

Además, estudios transversales en individuos mayores con DT2 demostraron que la activación noradrenérgica y la acción de los glucocorticoides afectan la activación cerebral en la región frontal, durante una tarea de MT (Chen et al., 2014), en respuesta al estrés (Patel et al., 2000; Schoofs et al., 2008). Es evidente que el estrés afecta a la DT2, así como que algunos procesos cognitivos, específicamente MT, pueden verse afectados en la población con esta enfermedad. Hasta donde sabemos, no hay estudios previos que hayan investigado la implicación del estrés agudo en la MT en pacientes con DT2. Por lo tanto, se necesita más investigación para comprender esta asociación.

Es bien conocido que los eventos estresantes, como consecuencia de la liberación de hormonas y neurotransmisores, explicados anteriormente, influyen en las áreas temporal y prefrontal del cerebro, áreas de recuperación de información (Joëls y Baram, 2009). La respuesta alterada del cortisol ante un estrés crónico y/o agudo, puede afectar a la memoria dependiente del hipocampo (Lupien et al., 1997). En esta línea, Dos Santos et al., (2017) demostraron un rendimiento alterado en la MD tras un estresor agudo en personas mayores sanas. En pacientes con DT2 también se ha demostrado un peor rendimiento en MD (Awad et al., 2004).

Hasta donde sabemos, no se ha investigado la implicación del estrés agudo en la DT2. Por lo tanto, se necesitan más investigaciones para comprender la participación del estrés en la recuperación de información en pacientes DT2.

Ciclo diurno del cortisol y función cognitiva en la DT2

Se ha demostrado una secreción circadiana alterada del cortisol en la DT2, en comparación con individuos sanos (Lederbogen et al., 2011). Para estudiar el ritmo circadiano del cortisol, se construye una curva de cortisol diurno mediante recolección de saliva cronometrada, como se mencionó anteriormente. Esta curva de estudio incluye el CAR y el índice DCS, entre otros.

Con respecto al CAR, se encontró un CAR aplanado asociado con la DT2 (Bruehl et al., 2009; Panagiotou et al., 2020), aunque otros grupos no encontraron diferencias (Hackett et al., 2014; Carvalho et al., 2015). La relación entre el cortisol y las funciones cognitivas no ha sido apenas estudiada. Por ejemplo, con respecto a la MT, Reynolds et al. (2010) informaron de una asociación entre una peor MT y niveles plasmáticos de cortisol más altos por la mañana. Por el contrario, Bruehl et al. (2007) no encontraron diferencias en la MT entre la población con DT2 y la población sana. Hasta donde sabemos, no hemos encontrado estudios que relacionen el CAR con la MT en pacientes con DT2. Si nos centramos en otros dominios cognitivos, como la MD, no se ha descrito ninguna asociación entre el CAR y el impacto en la diabetes. En cuanto a la FE, Bruehl et al. (2007) encontraron que la FE no estaba asociada con los niveles plasmáticos de C (C basal y AUC), pero no utilizaron el índice CAR en su investigación.

En cuanto a la DCS, se ha observado una DCS aplanada en la DT2 (Hackett et al., 2014; Lederbogen et al., 2011). Sin embargo, en el estudio realizado por Vreeburg et al. (2009), no se encontró ninguna relación entre la DCS y la DT2. En cuanto a la relación entre la DCS y la función cognitiva, Lee et al. (2007) encontraron una relación entre una peor función cognitiva en la DT2 y la FE y un aumento del cortisol salival.

Hasta donde sabemos, no existe literatura científica que aborde la relación entre el CAR y la DCS con la MD, MT y FE en individuos mayores con DT2.

Objetivo

La presente tesis doctoral investiga si la actividad del eje HHA y el SNS están relacionados con el rendimiento cognitivo en personas mayores con DT2. Se realizaron tres estudios con los siguientes objetivos e hipótesis:

Objetivos específicos e hipótesis de cada estudio

Estudio 1: El objetivo principal del primer estudio fue caracterizar la respuesta psicobiológica (es decir, afecto, Aas y cortisol salival) de hombres y mujeres mayores con DT2 a un estresor psicosocial agudo y sus efectos sobre el rendimiento de la MT, ya que ningún estudio previo ha investigado los efectos del estrés agudo sobre el rendimiento de la MT en personas mayores con DT2. Con base en investigaciones previas realizadas en personas sanas, esperábamos que los participantes en la condición de estrés mostraran aumentos en el estado de ánimo negativo, disminuciones en el estado de ánimo positivo y niveles más altos de C y Aas que los participantes en la condición de control. Además, esperábamos un efecto de deterioro del estrés sobre el rendimiento de la MT de acuerdo con el menor rendimiento de la MT encontrado en personas con DT2.

Estudio 2: Los objetivos del segundo estudio fueron investigar la respuesta psicobiológica (es decir, afecto, Aas y cortisol salival) a un estrés psicosocial agudo en personas mayores con DT2 en comparación con un grupo de participantes sanos de la misma edad y sexo, y explorar el impacto del estrés agudo en el rendimiento de recuperación de la MD. De acuerdo con estudios previos en población sana, planteamos la hipótesis de que la respuesta psicobiológica será mayor en la condición de estrés y, específicamente, la respuesta del cortisol de los participantes con DT2 será más pronunciada en comparación con los participantes sanos. Además, de acuerdo con hallazgos previos en personas mayores sanas, no esperábamos efectos del estresor agudo en el rendimiento de recuperación de la memoria.

Estudio 3: Los objetivos del tercer estudio fueron investigar el ciclo diurno del cortisol y el rendimiento cognitivo en la DT2, y analizar las relaciones entre dos índices del ciclo diurno del cortisol (CAR y DCS) y el rendimiento cognitivo (MD, MT y FE). También exploramos el papel de la depresión en las relaciones entre los índices de cortisol y los dominios cognitivos. Para ello, en este estudio, los pacientes con DT2 se compararon con personas mayores sanas. Con base en estudios previos, esperábamos que el grupo con DT2 tuviera un CAR y DCS más planos, así como un peor rendimiento cognitivo en comparación con los controles. Además, tanto para los grupos con DT2 como para los sanos, anticipamos que un CAR de mayor magnitud se asociaría con un peor rendimiento en la DT2, pero, al mismo tiempo, con un mejor rendimiento en la MT y la FE. Además, planteamos la hipótesis de que un DCS más plano se asociaría con una peor MD en DT2. Sin embargo, no teníamos suposiciones a priori sobre la asociación entre la MT y la FE con la DCS en la población con DT2. Finalmente, esperaríamos que la mayor depresión encontrada en los participantes con DT2 aumentara la fuerza de la relación entre los índices de cortisol y el funcionamiento cognitivo.

Metodología

Descripción general de los participantes

La muestra de participantes con diagnóstico médico de DT2 fue remitida por sus médicos. La muestra de participantes sanos procede de un programa de estudios de la Universitat de València para personas mayores de 55 años. Los criterios de exclusión fueron: fumar más de 10 cigarrillos al día, consumo de fármacos relacionados con las funciones cognitivas y/o emocionales, abuso de alcohol u otras drogas, presencia de trastornos cardiovasculares, trastornos psiquiátricos o siendo reclutada en hospitales y clínicas médicas neurológicas y deficiencias visuales o auditivas. Además, se excluyeron aquellas personas sometidas a los efectos de anestesia general y que hubiesen vivido un acontecimiento estresante en el último año.

Procedimiento

Estudio 1: Este estudio fue diseñado para investigar la respuesta psicobiológica (estado de ánimo, alfa-amilasa y cortisol) a un factor estresante agudo (Trier Social Stress Test; TSST) y su efecto sobre el rendimiento de la memoria de trabajo en personas mayores diagnosticadas con DT2. Para esto, 50 participantes con DT2, de 52 a 77 años, fueron asignados aleatoriamente a una condición de estrés (12 hombres y 12 mujeres) o de control (12 hombres y 14 mujeres). Se midió el estado de ánimo, mediante la administración de un cuestionario de autoeficacia, varios ítems para medir la valoración situacional y la Escala de Afecto Positivo y Negativo (PANAS). Las respuestas fisiológicas (cortisol y alfa-amilasa salival) a las tareas se midieron a través de la recogida de 7 muestras de saliva a lo largo de la sesión. Además, los participantes completaron una prueba de MT antes y después de la tarea de estrés o de control. Se utilizaron dos muestras de sangre para medir la concentración de glucosa utilizando un sistema de monitoreo de glucosa.

Estudio 2: Este estudio fue diseñado para investigar el efecto del estrés agudo en la recuperación de la MD en participantes mayores con DT2, en comparación con población sana. Para ello, 100 participantes, entre 52 a 80 años (50 con DT2 y 50 participantes sanos), fueron asignados aleatoriamente a dos condiciones de forma contrabalanceada: DT2 (24 a la condición de estrés y 26 a la condición de control); sin DT2 (24 a la condición de estrés y 26 a la condición de control). En la primera sesión (adquisición), por la mañana, los participantes visualizaron 30 imágenes con componente emocional (10 positivas, 10 negativas y 10 neutras), un listado de 15 palabras (RAVLT) y dos historias (subtest del test Rivermead). Un día después, los participantes regresaron al laboratorio por la tarde, para realizar la segunda sesión (recuperación). La mitad de los participantes fueron expuestos a un estresor psicosocial agudo (TSST) (o a una tarea de control no estresante). Después de la tarea de estrés/control los participantes respondían varias preguntas para su valoración situacional. A continuación, se completaba la prueba de recuerdo libre y de reconocimiento de

las imágenes que habían visto el día anterior, la prueba de recuerdo libre con las palabras RAVLT y las historias de Rivermead. Durante la sesión de recuperación, los participantes de ambos grupos y condiciones proporcionaron siete muestras de saliva para medir los niveles de cortisol y alfa-amilasa.

Estudio 3: Este estudio fue diseñado para investigar las diferencias en el ciclo diurno del cortisol, entre personas mayores sanas y con DT2. Además, se evaluó si el ciclo diurno del cortisol se relacionaba de forma diferente con la DT2, la MT y la FE. Para ello, la muestra estuvo compuesta por 51 pacientes con DT2 (26 hombres y 25 mujeres) y 51 participantes sanos (26 hombres y 25 mujeres). Se evaluó el rendimiento en diferentes pruebas de memoria y función ejecutiva. La MD (aprendizaje, recuerdo a corto plazo y recuerdo demorado) se evaluó con el test de RAVLT y el subtest de Rivermead. La MT se evaluó con dos pruebas verbales: Digit Span y secuenciación de Letras y Números. Para evaluar la función ejecutiva se utilizaron el Trail Making Test (TMT-A y TMT-B) y el Stroop Color-Word Test. Se tomaron dos muestras de sangre capilar al inicio y al final de la sesión para medir las concentraciones de glucosa. Además, para medir el ciclo diurno del cortisol, los participantes proporcionaron 4 muestras de saliva durante 2 días laborables consecutivos. Las muestras se obtuvieron inmediatamente después de despertar, 30 y 45 minutos después de despertar e inmediatamente antes de acostarse.

Resultados

Los resultados del primer estudio mostraron que un estresor psicosocial (TSST) fue capaz de producir una respuesta psicobiológica característica en pacientes con DT2. A nivel subjetivo, el TSST se percibió como más estresante, frustrante, difícil y requirió más esfuerzo que la tarea de control. Los participantes en la condición de estrés percibieron que tenían menos capacidad para realizar la tarea con éxito. Además, el TSST provocó cambios de humor caracterizados por

disminuciones en el humor positivo y aumentos en el humor negativo. El TSST también provocó una respuesta de C clara en pacientes con DT2.

Los resultados del segundo estudio mostraron que los participantes con DT2 informaron de una respuesta psicobiológica similar a la de los participantes sanos. Además, el estrés no afectó a la recuperación de la memoria de imágenes, palabras o historias en los grupos con DT2 ni en los sanos. Independientemente de la condición, los participantes con DT2, en comparación con los participantes sanos, recordaron menos ideas de párrafos tanto en la sesión de adquisición como en la de recuperación y menos palabras inmediatamente después del aprendizaje, pero no 20 minutos o 24 horas después del aprendizaje. Por último, sólo en los participantes sanos, la reactividad del cortisol al factor estresante se relacionó negativamente de forma marginal con el recuerdo sin palabras.

Los resultados del tercer estudio no mostraron diferencias entre CAR y DCS entre personas con DT2 y sanos . Además, los pacientes con DT2 presentaron peor rendimiento cognitivo en MT, MD y FE, en comparación con los controles sanos. Se evidenció una relación entre un CAR más alto y una mejor flexibilidad cognitiva en los participantes sanos, pero no en los participantes con DT2. Respecto a la relación entre los índices de cortisol y el rendimiento cognitivo, encontramos una relación negativa significativa entre CAR e interferencia de Stroop en el grupo sano. No se mostraron asociaciones entre DCS y DM. Por último, exploramos si la depresión pudiera afectar las relaciones estudiadas, encontrando que un CAR más alto se relacionaba con una interferencia de Stroop más baja en niveles medios de depresión . Además, encontramos que una mayor DCS se relacionó con una mejor puntuación en RAVLT demorado en niveles bajos de depresión.

Las siguientes conclusiones generales se pueden extraer de los estudios incluidos en esta disertación:

1. El TSST es capaz de producir la respuesta psicobiológica previamente reportada en personas sanas (estado de ánimo, Aas y respuesta de cortisol), en pacientes con DT2 tratados médicamente.

2. La exposición al TSST no afectó el rendimiento de la memoria de trabajo en pacientes con DT2, como se describió previamente en personas sanas.

3. El rendimiento de la memoria de trabajo está relacionado con la interacción entre el eje HHA y el SNS.

4. El estrés no afectó la recuperación de la memoria para imágenes, palabras o historias en DT2 y personas mayores sanas.

5. La respuesta del cortisol al estresor agudo está negativamente relacionada con la recuperación de palabras, pero no de imágenes e historias, solo en adultos mayores sanos y no en personas mayores con DT2.

6. Los participantes con DT2 recordaron un número menor de ideas sobre historias en la sesión de adquisición y recuperación en comparación con los participantes sanos, pero no se encontraron diferencias grupales en palabras ni imágenes.

7. Los adultos mayores con DT2 informaron un peor desempeño cognitivo (es decir, MT, MD y FE) en comparación con los individuos sanos.

8. Los participantes con DT2 mostraron un ciclo de cortisol diurno similar al de las personas mayores sanas.

9. El patrón de cortisol diurno, como reflejo del funcionamiento basal del eje HHA, no pareció estar significativamente relacionado con el desempeño cognitivo deteriorado observado en los participantes con DT2.

10. La relación entre un CAR más alto y una mejor flexibilidad cognitiva (interferencia de Stroop) se confirmó en los participantes sanos, pero no en los participantes con DT2.

11. Cuando se considera la depresión, se observa una relación negativa entre CAR e interferencia de Stroop para niveles bajos y medios de depresión.

12. Finalmente, se encontró una relación positiva entre DCS y RAVLT demorado para niveles bajos de depresión.

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