A Case-Control Study Investigating Spectral Power in the Brain During an Affective Shifting Task: A Cognitive Performance in Adolescents with and without Type 2 Diabetes (CPAT2D) Study

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Preface

I acknowledge that I am the sole author of this thesis. I confirm that I collected all data for this project, in collaboration with Irina Podinic and Kaeshan Elamurugan, supervised by PhD student Caroline Dutil, Dr. Jean-Philippe Chaput, and Dr. Anthony Carlsen. I performed all my data cleaning and preparation using the equipment from Dr. Stuart Fogel's lab and conducted all my analyses myself using SPSS. I was a member of the Healthy Active Living and Obesity research group for five years, starting with my work as a research assistant on Caroline Dutil's doctoral thesis project entitled Sleep Manipulation in Adolescents at Risk of Type 2 Diabetes (SMART2D). The SMART2D project informed the Cognitive Performance in Adolescents with Type 2 Diabetes project, for which my thesis focuses on a subset of data. I was able to develop my research skills and knowledge through my years working on the SMART2D project, which allowed me to confidently conduct my own experiment. Testing days for both studies were quite intense, usually lasting up to eight hours a day and involving complex scientific techniques such as testing blood glucose levels, administering questionnaires on mental health, and providing engaging and interactive testing sessions. I believe the findings of my research study will yield an important and interesting research article, which will be published.

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Abstract

Paediatric cases of type 2 diabetes (T2D) are becoming more common as rates of obesity rise in tandem. Literature has suggested that T2D is associated with poorer cognitive performance and lower overall brain health compared to healthy controls. Performance differences across several cognitive domains have previously been investigated by looking at changes in spectral power using electroencephalography (EEG). The purpose of this study was to determine if adolescents with T2D have lower spectral power in the theta, alpha, and beta bands during a complex cognitive and motor task, compared to those without. This question was addressed using a case-control study, comparing 11 adolescents with T2D (cases) to 11 who are apparently healthy and 11 who are at-risk of T2D, for a total of 22 controls. Cognitive performance was measured using an affective shifting task in tandem with EEG. Results did not reveal any significant differences between groups, which suggests that the adolescent brain may be still producing brain waves at a comparable rate to adolescents without T2D. Further research is needed to better understand at what point spectral power differences appear.

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List of Abbreviations

AH	Apparently Healthy
ANOVA	Analysis of Variance
At-Risk	At-Risk of Type 2 Diabetes
AST	Affective Shifting Task
BMI	Body Mass Index
CHAL	Center for Healthy Active Living
CHEO	Children's Hospital of Eastern Ontario
CPAT2D	Cognitive Performance in Adolescents with Type 2 Diabetes
EEG	Electroencephalography
F	Block of the affective shifting task where the target stimulus is an image of Food.
FFT	Fast Fourier Transform
Go-F	Go-F refers to moving the right wrist in a ballistic, 45-degree angle in response to an image of food where the target is food.
Go-O	Go-O refers to moving the right wrist in a ballistic, 45-degree angle in response to an image of an object where the target is objects.
MEG	Magnetoencephalography
NG-F	NG-F refers to not moving the right wrist in response to an image of food where the go-target is objects.
NG-O	NG-O refers to not moving the right wrist in response to an image of objects where the go-target is food.
O	Block of the affective shifting task where the target stimulus is an image of an Object.

SMART2D	Sleep Manipulation in Adolescents at Risk of Type 2 Diabetes
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
WHO	World Health Organization

Chapter 1: Introduction

Over the past twenty years, diagnoses of type 2 diabetes mellitus (T2D) have spiked in child and adolescent populations, in tandem with rising rates of obesity (Amed et al., 2010). A research study by Amed and colleagues (2010) also found that approximately 90% of new youth-onset T2D cases occur during adolescence. When studying this statistic further, researchers discovered that early pubertal onset is a primary risk factor for developing T2D before adulthood (Ohlsson et al., 2020). In adults, the link between T2D and brain health has been clearly demonstrated, with findings showing that adults living with T2D are 50% more likely to develop Alzheimer's disease and 150% more likely to develop vascular dementia than those without (Amed et al., 2010). However, the link in adolescents is much less clear due to the significant lack in research.

Several preliminary research studies have discovered key structural brain differences in adolescents with T2D compared to those without, such as decreased total brain volume, and decreased white and grey matter volumes in areas of the brain such as the prefrontal cortices (Yau et al., 2010, & Bruehl et al., 2011, & Rofey et al., 2015, & Nouwen et al., 2017). Furthermore, many of these regions are undergoing rapid maturation during adolescence. Specifically, rapid maturation occurs in the prefrontal cortex, posterior parietal cortex, and superior temporal cortex, which are responsible for decision making, impulse control, and working memory (Larsen & Luna, 2018).

Several studies have emerged examining the impact of T2D on cognitive function and have yielded concerning findings. For example, a recent meta-analysis revealed that adolescents with T2D consistently perform more poorly on cognitive tasks than their otherwise healthy counterparts (van Duinkerken & Ryan, 2020). Considering these group differences, an underlying question remains: Is this pattern of poorer cognitive performance related to the structural brain differences? Moreover, to what extent do structural brain differences affect other neurological processes, like spectral power?

Electroencephalography (EEG) is a technique that allows researchers to capture brain wave densities during cognitive tasks, offering a unique opportunity to observe and compare spectral power between different population types. Although the literature examining T2D in adolescents is sparse, study findings have discovered that adolescents with type 1 diabetes (T1D) have lower spectral power in the higher frequencies (i.e., alpha, beta, and gamma) during a cognitive task than those without T1D (Hyllienmark et al., 2005). Researchers have also found a relationship between the densities of certain spectral frequencies and body weight, with several studies finding that adults with obesity and adults with food addictions have increased beta power compared to healthy-weight adults (Hume et al., 2015 & Imperatori et al., 2015).

When discussing cognitive function, there are other variables that must be considered. For example, without controlling for sleep, it would be impossible to determine whether T2D or sleep duration is responsible for spectral power differences. One out of every three Canadian adolescents is not meeting the sleep duration recommendations based on age, and it has been well established in the literature that cognition and cognitive performance are heavily influenced by sleep (Roberts et al., 2017).

The potential significance of a case-control research project comparing spectral power in adolescents with T2D to those without offers valuable insight into the role of T2D on the developing brain. A preliminary, observational study can provide the first evidence of possible neurological differences in alpha, beta, and theta wave densities found in adolescents with T2D compared to adolescents without. The results from a study like this can have clinical significance because the impact of T2D on the developing adolescent brain remains largely unknown. While there are studies that have focused on structural brain differences as well as cognitive performance, there are no studies to date comparing spectral power (more specifically, theta, alpha (low and high) and beta (low and high) waves) between adolescents with T2D and those without. Given that adolescence marks a period of great change, both emotionally and physically, it is important to close this gap in the literature in order to better inform the scientific community and future research in the area. Ultimately, the long-term goal is to impact practice and guidelines.

Chapter 2: Literature Review

Type 2 Diabetes

The World Health Organization (WHO) defines diabetes as a “chronic, metabolic disease characterized by elevated blood glucose” for which there are two types: T1D, which is where the pancreas does not produce enough insulin, and T2D, where the body becomes resistant to insulin (WHO, n.d., *diabetes*, para 1). Historically, paediatric cases of diabetes have been predominantly T1D; however, the increasing prevalence of child and adolescent obesity has subsequently shown a spike in cases of T2D before adulthood (Hannon et al., 2005). Although T2D is a metabolic disorder, it has been linked with a multitude of comorbid disorders in adults. For example, adults with T2D are 50% more likely to develop Alzheimer’s disease and 150% more likely to develop vascular dementia than otherwise healthy adults (Amed et al., 2010). Although research in adults with T2D is extensive, there are few research studies examining the effects of T2D on the adolescent brain.

In the literature, four research papers published within the last two decades have identified key structural differences in the brains of adolescents with and without T2D. For example, a research study by Yau and colleagues (2010) conducted magnetic resonance imaging (MRI) on 18 adolescents with obesity and T2D and 18 adolescents with obesity but without T2D. Their findings revealed a significant volumetric reduction in white matter volumes, and increased amounts of cerebral spinal fluid in both the frontal lobes and the entire brain in adolescents with T2D (Yau et al., 2010). This research finding suggests that it is the metabolic disorder itself that is responsible for the reductions in brain volume as opposed to excess weight alone. A major weakness of this study, however, is that they did not include any controls without obesity. Although this may appear insignificant, adolescents with obesity are likely to show minor resistance to insulin already, making them not a completely unique comparator for adolescents with T2D (Yau et al., 2010).

A secondary research study by Rofey and colleagues (2015) compared three groups of fifteen adolescents: five with an official diagnosis of T2D, five matched on obesity but without T2D, and five healthy controls. Each adolescent completed an MRI of their brains, specifically examining grey matter volumes and white matter integrity (Rofey et al., 2015). Results from this study revealed that the healthy control adolescents had a significantly greater volume of grey matter in the caudate nucleus than the other two groups, and that both the healthy controls and adolescents with obesity had greater thalamic volumes than adolescents with T2D (Rofey et al., 2015). Despite a lack of statistical difference in any other parts of the brain, clinical differences were found in the hippocampus, amygdala, putamen, nucleus accumbens, and pallidum (Rofey et al., 2015).

The results from the white matter integrity scan revealed similar findings, indicating that both adolescents with obesity and adolescents with T2D had reductions in white matter integrity, compared to the healthy controls (Rofey et al., 2015). White matter is known to speed up neural firing and optimize the brain's processing speed, therefore having less of it indicates that the brain is not maximally efficient (Fields, 2010). Despite such significant findings, this study is also limited by its small sample size. This limitation is further questioned when considering that both adolescents with T2D and adolescents with obesity had significantly reduced volumes of grey matter. Without a larger sample size of each group, it is impossible to tell if these structural brain differences are attributable to excess weight or to diabetes.

Similar research findings were discovered by Bruehl and colleagues (2011), who did a case-control, MRI study comparing 18 adolescents with obesity and T2D to 18 adolescents with only obesity. The purpose of this study was to determine if significant volumetric differences exist between adolescents with and without T2D, particularly in regions of the brain such as the frontal lobe and hippocampus (Bruehl et al., 2011). Results from this study revealed that adolescents with T2D have significantly smaller hippocampal and frontal lobe volumes compared to non-T2D adolescents, as well as greater cerebral atrophy overall (Bruehl et al., 2011). Although a strength of this study was the MRI capability to capture accurate spatial resolution, it also lacks temporal resolution, therefore a study using EEG would provide further insight into how these structural brain differences might impact cognitive function temporally (Bruehl et al., 2011).

A 2017 study by Nouwen and colleagues yielded similar neurological findings when they compared adolescents with T2D to adolescents without. Their study compared three groups of adolescents: 15 with an official diagnosis of T2D, 21 adolescents with obesity, and 22 controls for which all adolescents participated in an MRI scan to determine if structural brain differences existed between groups (Nouwen et al., 2017). Consistent with previous research findings, adolescents with T2D had significantly smaller grey matter volumes, particularly in the putamen and caudate nucleus than either of the control groups (Nouwen et al., 2017). Adolescents with obesity also had significantly smaller grey matter volumes than the control adolescents, specifically in the right hippocampus, and left putamen, caudate nucleus, and amygdala (Nouwen et al., 2017). Interestingly, lower grey matter volumes are also associated with a higher BMI, which presents difficulties when interpreting research findings, as both adolescents with obesity and adolescents with T2D presented with lower volumes (Nouwen et al., 2017). A major limitation of this study is that cognitive function was not assessed during the testing. Research literature has already demonstrated that adolescents with T2D experience mild cognitive impairments; however, this finding has not been taken in the context of the structural brain differences (Nouwen et al., 2017). In order to better understand the role of the brain differences in adolescents with and without T2D, cognition must be assessed in tandem with population comparison.

Although preliminary research findings have presented evidence of key structural brain differences between adolescents with and without T2D, the disease itself also greatly implicated cognitive function. For example, a recent study revealed that 18-year-olds with T2D perform more poorly than their healthy counterparts on tasks assessing intellectual functioning, verbal memory, and psychomotor efficiency (van Duinkerken & Ryan, 2020). One possible proposed reason for this is that a glucose metabolism imbalance causes poorer cognitive performance (van Duinkerken & Ryan, 2020).

The aforementioned study also briefly examined structural brain differences in adolescents. At a neurobiological level, there have been notable differences in frontal lobe and overall white matter volume in the brains of people with T2D, which would also impact cognitive function (van Duinkerken & Ryan, 2020). Furthermore, there is also a higher prevalence of infracts in people suffering from T2D, meaning brain tissue death due to inadequate blood flow (van Duinkerken & Ryan, 2020). Given that this research is so novel, it presents a large area requiring further research, as T2D has been linked with such serious diseases in adulthood and is presenting with brain differences in adolescents as well. The more that is understood about the impact of these brain differences, the more that can be done at an intervention level to prevent further damage.

Obesity

The World Health Organization's (WHO) definition of overweight and obesity depends on the age and sex of a person. For youth, the WHO defines being overweight as any child or adolescent (ages 5-19) who is over one standard deviation above the Growth Reference median (de Onis et al., 2007). For those with obesity, it is greater than two standard deviations above the Growth Reference median (see Appendices C&D). As mentioned above, both T2D and obesity affect the brain structurally as well as cognitive function, which has been supported by many research studies. For example, a systematic review by Wang and colleagues (2016) supported the notion that obesity in general is associated with poorer cognition, poorer motor control, and altered brain plasticity. Furthermore, obesity has been linked with lower verbal fluency, and delayed recall (Wang et al., 2016).

In adolescence, obesity has been found to have the largest impact on attention and cognitive functioning, both of which are of vital importance (Wang et al., 2016). Furthermore, earlier exposure to a high-fat diet has been found to relate to impairments in long-term spatial memory difficulty (Wang et al., 2016). A major limitation of this systematic review is that none of the effect sizes were mentioned, making it difficult to determine the actual significance of the research findings. With a large enough sample size, small differences in memory between groups become statistically significant. A case-control study will aid in clarifying the actual group differences, as each population type can be compared objectively.

A second study by Sweat and colleagues (2017) compared 11 domains of cognitive functioning between otherwise healthy adolescents and those with obesity (n=162, age=19.5 ± 1.5 years old). Results indicated that adolescents with obesity had significantly slower processing speeds than the healthy adolescents in four of the 11 categories assessed. However, there were no significant differences noted in executive functioning between the two groups (Sweat et al., 2017). This suggests that although some functions are compromised with obesity, the brain is able to compensate and preserve executive functioning (Sweat et al., 2017). Although this may appear to be a positive finding, it nonetheless indicates that the brains of adolescents with obesity are working harder than they should be to complete the same tasks.

A critique of this study is that Sweat and colleagues (2017) failed to control for sleep during their experiment, which limits the strength of the relationship that can be drawn between adolescence and cognition. It is well known that sleep is vital for both adolescent development and cognitive function (Dutil et al., 2018 & Galvàn, 2020). It is therefore paramount to control for this variable, as it can act as a confounder when interpreting research findings.

Adolescent Populations

The WHO defines adolescence as a transitional period in the middle of childhood and adulthood; more specifically, beginning at age 10 until age 19 (WHO, n.d.). According to Sawyer and colleagues (2018), adolescence represents the period between childhood and adulthood, including not only physical transformations, but also changes in social roles. Despite some commonalities, such as age period, there are many other factors that can affect the developmental experiences of different groups of adolescents; a major one being weight. Over the last forty years, the prevalence of excess weight and obesity in Canada has changed from one in four adolescents to one in three (Rao et al., 2016). Obesity is also a significant predictor of T2D in adolescents (Rao et al., 2016). For this reason, it is important to examine cognition between groups, as not all adolescents have shared experiences.

Cognition – Brain Waves

Although there is a plethora of validated questionnaire measures of cognition, it can also be assessed using brain imaging techniques such as electroencephalography (EEG) when paired with a cognitive task. This type of technology can capture different brain waves in real time using electrodes. The arrangement uses a 10-20 system, which standardizes the placement of electrodes on the scalp (Silverman, 1963).

The brain itself has five primary types of waves: delta, theta, alpha, beta, and gamma waves, with each corresponding to different cognitive functions (Malik & Amin, 2017). Delta waves (0.5-4Hz) are high amplitude and low frequency and are indicative of deep sleep (Amin & Malik, 2016). For the purposes of this study, I will not be looking at delta waves. My primary focus for this project was to look at higher frequency brain waves, which are indicative of more complex cognitive function. Additionally, given that delta waves are mostly present during sleep, and all my participants were awake during the testing sessions, I am less interested in their presence during this experiment. I will therefore not be discussing them in the review of the literature.

Theta Waves

Theta waves are the second slowest brain wave and represent all spectral power between the 4.5 and 7Hz range. Higher theta waves have also been linked to different cognitive functions depending on age (Amin & Malik, 2017). For example, in children, higher density theta waves indicate drowsiness, whereas theta waves in adults are associated with working memory and attention (Amin & Malik, 2013). Theta wave density is also impacted by other factors, like cognitive demand.

A group of researchers paired a go/no-go task with EEG in a group of healthy adults to measure any changes that may occur throughout the experiment (Funderud et al., 2012). They found that event-related spectral power was lower in the theta frequency during the "go" trials, compared to the "no-go" trials. Ergen and colleagues (2014) also studied spectral power in adults during a Stroop task and found that theta waves were higher during incongruent trials compared to congruent trials, which suggests that a higher cognitive demand is required when the stimulus presented is more complex. Isabella and colleagues (2021) also examined spectral power during a complex cognitive task by pairing magnetoencephalography with a go/switch task. They tested healthy adults and found that theta waves peaked in the brain just before response time (Isabella et al., 2021). The literature has shown a clear relationship between theta waves and cognition; however, the findings are somewhat divided in adults when it comes to theta peaks. For this reason, as well as the clear gap in literature studying adolescents, further research is needed.

Another known factor to impact theta waves is population type, with a key population being adults with obesity, mild cognitive impairments, and diabetes. Imperatori and colleagues (2015) conducted an interesting study where they had a group of adults who were overweight with three or more clinical signs of food addiction, and a group of adults with obesity who had two or less signs of food addiction. Resting spectral frequencies were measured followed by a sip of a chocolate milkshake and then a neutral stimulus (allowing for time to return to baseline in between) (Imperatori et al., 2015). Their findings suggested that addiction to food is related to a higher resting theta, compared to healthy adults (Imperatori et al., 2015). Although these findings provide insight into the relationship between food and theta waves, there is still much to be learned about other population types, such as adolescents or those with T2D.

Another observational study by Hyllienmark and colleagues (2005) compared adolescents with T1D to a group of healthy controls. They also recorded participants at a resting state, comparing spectral power with eyes open and eyes closed (Hyllienmark et al., 2005). They found that adolescents with T1D had increased theta waves compared to healthy adolescents (Hyllienmark et al., 2005), which is significant because theta waves are commonly known to fall under the "slower" wave frequencies and are not always associated with complex cognitive function. However significant, the question remains if these findings are present among other populations, such as adolescents with T2D.

While there are gaps in literature surrounding EEG and adolescents with T2D, there have been some studies that look at adults with T2D. For example, Benwell and colleagues (2020) compared resting EEG and cognitive performance (using neuropsychological tests) between healthy adults, adults with T2D and adults with Alzheimer's disease. They found that adults with T2D had higher densities of slower brain waves than a healthy control group as well as performing more poorly on the tests that assessed learning and memory (Benwell et al., 2020). It is clear, through the literature, that there is still much that is unknown regarding the impact of T2D on the developing brain, especially in the pediatric population.

Alpha Waves (low and high)

The next waves of interest are alpha waves, which are low amplitude and high frequency. They are typically associated with active brain processes such as working memory and memory retention, making them one of several waves of possible interest when discussing cognition and cognitive performance (Tuladhar et al., 2007). Researchers have also found that alpha waves tend to peak in two different regions: in a lower frequency (8-10Hz) and at a higher frequency

(11-13Hz) (Olejarczyk et al., 2017, & Imperatori et al., 2015, & Funderud et al., 2012). Low alpha waves have been associated with attention, whereas high alpha waves are associated with sensory and semantic information (Klimesch, 2005). Furthermore, people with high alpha peaks tend to have faster reaction times, whereas those with low alpha peaks tend to have slower reaction times (Klimesch, 2005). Furthermore, researchers have found higher alpha waves to be related to attention as well as inhibition (Cooper et al., 2016, Funderud et al., 2012, and Kuo et al., 2022).

Cooper and colleagues (2016) examined spectral power during an oddball task (using a go/no-go paradigm) in healthy adults. They found that alpha waves, specifically in the parietal region of the brain, decreased during the switch tasks (Cooper et al., 2016).

Funderud and colleagues (2012) also examined spectral power in healthy adults during a go/no-go task. Their findings suggested that alpha waves were higher during the no-go trials compared to the go trials (Funderud et al., 2012). Kuo and colleagues (2022) also studied spectral power during a go/no-go task and found that alpha waves were higher during the no-go trials compared to the go trials. A strength of this study is that they chose to study a paediatric population, for which there is much that is still unknown; however, it was a special population of children undergoing surgery for epilepsy, so it is unknown if spectral power differences could also be found in other paediatric populations (Kuo et al., 2022).

Like theta waves, alpha waves are also impacted by mitigating factors like obesity and diabetes. The study by Hyllienmark and colleagues (2005) (discussed more fully under theta waves) found an overall reduction in fast waves (including alpha waves) in adolescents with T1D compared to the control group. This finding is not novel; researchers dating back sixty years have reported that children with diabetes have significantly lower power in the alpha band frequency compared to healthy controls (Eeg-Olofson and Peterson, 1966). A limitation of this study, however, is that the type of diabetes was not specified and so it is hard to draw meaningful conclusions on the impact of the disease on the developing brain.

Benwell and colleagues (2020) published alarming findings from their study examining resting spectral power in adults with Alzheimer's disease, T2D, and healthy controls. They found that adults with both Alzheimer's disease and T2D had lower alpha power compared to the healthy controls (Benwell et al., 2020). Given that T2D is related to increased chances of developing Alzheimer's disease, this finding is particularly concerning (Amed et al., 2010).

A strength of this study was that they also used cognitive measures to assess brain function and found that adults with T2D consistently performed more poorly on tasks of learning and memory, compared to the healthy controls (Benwell et al., 2020). It is useful to know, however, at what point these differences start to appear and therefore studying a paediatric population would provide much needed insight into the trajectory of T2D on the brain.

Although there are numerous findings suggesting diabetes is related to lower alpha power, results are also mixed. For example, Gallardo-Moreno and colleagues (2020) found that alpha waves were higher in children with T1D compared to healthy controls. Given the inconsistencies, further research is needed to better understand the impact of metabolic diseases, such as diabetes, on spectral power. The literature gap is widened when considering the lack of research focusing on T2D.

Beta Waves (low and high)

Beta waves are structurally similar to alpha waves in the sense that they are also low amplitude, high frequency, and are indicative of higher order mental processing like somatosensory and motor functions (Pfurtscheller et al., 1996, & Engel & Fries, 2010). Similar to alpha waves, beta waves also encompass a large spectral frequency (14-30Hz) which can result in washing out effects, therefore one method of studying beta waves is to split them into low beta (14-20Hz) and high beta waves (21-30Hz). This methodology was implemented by Funderud and colleagues (2012); however, it is also empirically based in the literature.

As with theta and alpha waves, one way to measure beta power is by using EEG paired with a cognitive task. For example, Lim and colleagues (2019) studied spectral power in young adults during a phase of concentration and a phase of immersion. For the concentration, they had participants focus on a dot on a screen whereas for immersion, they had participants focus on playing a video game (Lim et al., 2019). Their results found that beta waves increased during concentration, compared to baseline, and increased even more during the immersion task, compared to baseline (Lim et al., 2019). As with Engel and Fries (2010), Lim and colleagues (2019) also found that beta waves increased during motor preparation.

Given that many complex cognitive tasks, such as go/no-go tasks, also involve motor function, these waves are of interest when examining cognitive function and cognitive load. Güntekin and colleagues (2013) also studied the role of beta waves in cognitive function using a complex cognitive task called a visual oddball paradigm. They found that beta waves tend to peak during “target” trials (which would be similar in cognitive demand to a “go” trial) compared to non-target trials (Güntekin et al., 2013).

Spectral power has also been found to differ during a cognitive task, depending on the stimulus type, suggesting there could be an interaction between cognition, population type, and stimulus saliency. For example, Hume and colleagues (2015) compared EEG during a Stroop task between women of a healthy weight, women who were overweight, and women with obesity. Their results indicated that overweight women had increased beta activity in response to the food cues compared to the neutral cues (Hume et al., 2015).

Beta waves have also been found to differ between population types. For example, Hyllienmark and colleagues (2005) found that adults with Alzheimer's disease had lower beta power compared to healthy controls. These researchers also found that adolescents with T1D had lower beta power than healthy controls (Hyllienmark et al., 2005).

This review of the literature has clearly demonstrated several inconsistencies in research findings, which further supports the demand for more research into this subject, especially as it pertains to adolescent brain development.

Salience of Stimuli

Research has found that not only is spectral power impacted by sleep and population type, but also by emotional salience. For example, Funderud and colleagues (2012) compared brain waves during a go/no-go task between a neutral stimulus (a picture of a car) and an emotionally salient stimulus (a picture of a dog). Their findings revealed that spectral power was significantly higher during the emotionally salient trials compared to the neutral trials (Funderud et al., 2012). Another study by Nijs and colleagues (2008) compared spectral power in adults while looking at pictures of food versus neutral stimuli. They found that images of food elicit higher spectral power compared to the neutral stimuli, more specifically in the posterior and central regions of the brain (Nijs et al., 2008). This finding is particularly relevant, considering the Muele et al. (2014) task is made up of images of both food and objects. The findings of Nijs and colleagues (2008) were further supported by Luo and colleagues (2009) who found that event-related synchronizations in the gamma band frequency were related to emotionally salient stimuli. Altogether, the literature is consistently demonstrating that spectral power is impacted by many elements of a complex cognitive task and the design of Muele and colleagues (2014) is particularly unique because it allows for the observation and comparison of multiple facets at the same time. It is therefore an optimal task to use for a case-control study.

Regions of Interest

Given that most EEG technology is composed of many electrodes, it does not always make sense to look at all channels, especially since the electrodes reside over different regions of the brain. For example, during a complex cognitive task, there is heavy demand on attention, inhibition, decision-making, and motor control (Kolb and Whishaw, 2014). It is commonly known that many of those cognitive functions are frontal-lobe dominant, making that region of particular interest when studying spectral power. Secondly, for cognitive tasks that also utilize motor skills, such as more complex go/no-go tasks (Muele et al., 2014), examining spectral power in the electrodes that are on top of the motor cortex and SMA can be of particular interest (Kolb and Whishaw, 2014). Given the specific regions of the brain known to be activated during complex cognitive and motor tasks, it is scientifically sound to only focus on those regions rather than the whole brain. Additionally, given the complex nature of EEG technology, it can be difficult to maintain perfect integrity at all electrode sites for the duration of a cognitive task. For this reason, it can be better to not only focus on the electrodes corresponding to specific regions of the brain, but also the electrodes that had the highest success rate in terms of consistently and accurately capturing spectral power. Additionally, focusing on this region of interest was previously used by Funderud and colleagues (2012), yielding significant findings.

Sleep

The covariate of sleep is important to examine because not only is it crucial for adolescent development, but it also impacts each dependent variable individually (Galván, 2020 & Chaput et al., 2016). To begin, it is important to examine the role of sleep during adolescence. According to the 24-hour movement guidelines, which are a set of principles detailed by the Canadian Society for Exercise Physiology, adolescents aged 14 to 17 years require eight to ten hours of sleep per night (Roberts et al., 2017 & Chaput et al., 2016 & Tremblay et al., 2016). A 2017 study by Roberts and colleagues revealed that one third of all Canadian adolescents are not meeting this guideline. This is of great concern because the brain is undergoing major changes in areas responsible for cognition, emotion, and learning during this time period (Galván, 2020 & Chaput et al., 2016). Furthermore, human growth hormone is released during sleep, which is vital during developmental periods such as adolescence (Leproult & Copinschi, 2015).

There are also several neurological domains that depend heavily on sleep for healthy development, such as executive functioning, risk-taking behaviour and emotion regulation (Galván, 2020). Of particular importance is the role of sleep duration on cognition as it pertains to emotion. A recent systematic review revealed that short sleep duration relates to increased emotional reactivity during adolescence (Dutil et al., 2018).

Sleep duration also impacts spectral power in several ways. First, a study by Tassi and colleagues (2006) compared spectral power in adults who slept 8 hours versus those who slept 2 hours (sleep deprived). They found that adults who experienced sleep deprivation had significantly higher theta waves compared to those who slept for 8 hours (Tassi et al., 2006). A second study by Li and colleagues (2008) revealed that spectral power significantly decreased at the 40Hz frequency after 24 hours of sleep deprivation. It is therefore necessary to control for sleep as well when examining the role of diabetes on gamma wave density in adolescents.

Purpose, Hypotheses, and Potential Significance

Currently, most of the research examining the impact of T2D on cognition has focused on adult populations. The study findings of structural brain differences in adolescents with T2D brings into question the impact of paediatric T2D on the developing brain, including cognitive performance. Spectral frequencies like alpha, beta, and theta waves have been found to be associated with higher-level cognitive functions such as attention, motor preparation, working memory, and inhibition (Cooper et al., 2016, & Lim et al., 2019, Funderud et al., 2012). However, as mentioned, spectral power is also impacted by factors like obesity (Hume et al., 2015). It is therefore essential to account for obesity when considering a nested case-control study comparing spectral power in adolescents with T2D who also generally have obesity.

Given the previous literature discussing spectral power and cognition, the primary research question of this project was: do adolescents with T2D have differences in spectral power during an affective shifting task (measuring both behavioural inhibition and selective attention), compared to adolescents who are apparently healthy and adolescents with obesity? This type of task directly challenges selective attention, behavioural inhibition, and working memory (Muele et al., 2014).

There were several main hypotheses that were tested through this project. Firstly, based on previous literature, it was hypothesized that adolescents with T2D would have lower overall spectral power in all five spectral frequencies compared to the control group (composed of both apparently healthy adolescents and adolescents at risk of T2D) (Hyllienmark et al., 2005, Eeg-

Olofson and Peterson, 1966). This was based on previous research findings indicating that youth with T1D have been found to have lower spectral power compared to those without (Hyllienmark et al., 2005, Eeg-Olofson and Peterson, 1966).

Secondly, it was hypothesized that spectral power, specifically alpha and theta waves, would be higher during no-go trials compared to go trials for all groups (Funderud et al., 2012 & DeLaRosa et al., 2020, & Kuo et al., 2022). This was based on results from numerous studies, using a variety of complex cognitive tasks, which found that specifically alpha and theta waves were higher during no-go trials compared go trials (Funderud et al., 2012 & DeLaRosa et al., 2020, & Kuo et al., 2022).

Lastly, based on previous literature, it was hypothesized that spectral power for all groups would be higher for food (more salient stimulus) compared to objects (neutral stimulus) due to the increased demand on inhibitory control (Nijs et al., 2008, & Gallardo-Moreno et al., 2020, & Imperatori et al., 2015, & Luo et al., 2009).

As described in the above review of the literature, there are still many unknowns when it comes to both T2D and spectral power but also to cognitive tasks and spectral power. Where some researchers have consistently found significantly lower waves in special populations, others have found the opposite. Additionally, where some researchers have found brain waves to be higher during certain types of trials of cognitive tasks, others have found the opposite as well. Through an in-depth review of the literature, it was clear that there is still much that is unknown and is lacking in scientific knowledge. Therefore, the potential implication of this case-control study comparing spectral power between three types of adolescent populations is significant. The polarizing research findings on spectral power during complex cognitive tasks in tandem with the significant research gaps involving adolescents with T2D provides a compelling argument to conduct further research. This study is the first of its kind, providing much needed insight into the impact of T2D on spectral power. Results from this study can begin to close the gap in the literature on this subject and further scientific knowledge on the subject.

Chapter 3: Methods

Ethical Considerations

Ethics was obtained to conduct this study from both the Children's Hospital of Eastern Ontario (CHEO) and the University of Ottawa (see Appendices A and B). Informed consent was obtained from each participant prior to commencing any study protocols. Participants were compensated with $25 and 10 hours of community volunteering credit.

Overview of study design history

This project was part of a larger study titled Cognitive Performance in Adolescents with Type 2 Diabetes (CPAT2D). The CPAT2D study was also derived from a larger experimental study that manipulated sleep duration in high-risk adolescents, titled the Sleep Manipulation in Adolescents at Risk of Type 2 Diabetes (SMART2D) study. Funding for both projects was awarded to Dr. Jean-Philippe Chaput in 2019 and the customized equipment, software, and the cognitive tasks were provided and/or created by Dr. Anthony Carlsen and PhD candidate Caroline Dutil.

Participants

For this study, there were two control groups and one case group, each composed of n=11 adolescents between the ages of 13 and 18, making up a total of n=33. The case group was made up of adolescents with an official diagnosis of T2D (with medication adherence) and were compared to a group of adolescents with obesity but without T2D, and adolescents without obesity and any chronic illness who were apparently healthy. Each of these three groups were recruited differently, beginning with adolescents with T2D.

Adolescents with T2D were recruited through the Endocrinology clinic at the Children's Hospital of Eastern Ontario (CHEO) based on the physician recommendation of Dr. Stasia Hadjiyannakis. All participants in this group required an official diagnosis of T2D and a body mass index (BMI) >97th percentile based on age and sex, as defined by the World Health Organization (WHO). The at-risk for T2D adolescents with obesity were recruited through the Centre for Healthy Active Living as well as the CHEO Endocrinology clinic, as part of the SMART2D study. The at-risk determination was based on several factors: a BMI > 99th percentile, as defined by the WHO, based on age and sex, and one or more of the following criteria: dyslipidemia (HDL and/or total cholesterol), a diagnosis of polycystic ovarian syndrome, and/or a diagnosis of non-alcoholic fatty liver disease. A specific criterion to the participants from the SMART2D study was that they had to report sleeping (on average) between 6.5 and 8 hours per night. Given that sleep duration was one of the variables for which participants were matched, the other two groups of adolescents (those with T2D and those who were apparently healthy) also followed this inclusion criterion. As such, in addition to the sleep duration criterion, participants in all groups were also excluded if they had a history of psychiatric illnesses, untreated sleep apnea, or were taking medications that affected sleep.

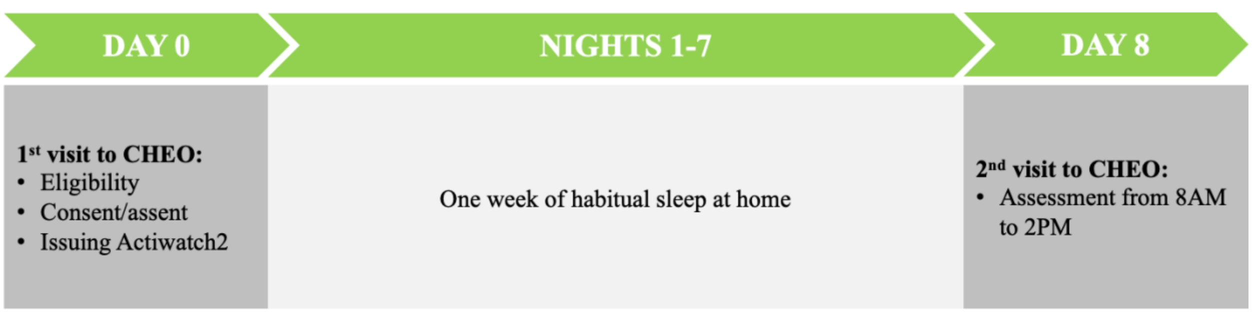
The adolescents without any chronic illness who were apparently healthy were recruited through social media and posters. Inclusion criteria for this group included a normal weight status, defined as a BMI >15th but <85th percentile based on age and sex, as defined by the WHO.

Participants were matched 1:1:1 between groups based on sex and/or gender, sleep duration, pubertal status, socioeconomic status, and school grade. Matching participants on multiple characteristics allowed for meaningful comparisons to be made, as it minimized the risk of mitigating factors affecting the analyses.

Study Design

The CPAT2D study was a case control study that successfully recruited 22 adolescents: 11 with an official diagnosis of T2D and 11 who were apparently healthy. The study involved a one-week commitment of the participants, with day one being a 1.5-hour, in-person, preliminary intake session to obtain consent and verify eligibility (see Figure 1). Once consent was obtained, participants completed several questionnaires that assessed eligibility and various lifestyle domains such as eating habits, sleep quality, pubertal status using the self-reported Tanner Scale (Emmanuel & Bokor, 2022), quality of life, and food preferences. Height and weight were also obtained during the intake session.

Figure 1. *Study timeline for the CPAT2D study.*



Note. This figure was adapted from materials developed by Caroline Dutil for the SMART2D study.

Prior to leaving the lab, all participants were issued booklets containing different kinds of logs. Adolescents with T2D (case group) were issued a week-long sleep log, a 3-day food log, and a week-long T2D-related medication log to verify medication adherence. Both control groups had the same logs, including a week-long sleep log, a 3-day food log, and a week-long caffeine log. Adolescents in all groups were also issued an Actiwatch 2 (a sleep accelerometer that monitored sleep) which was used to measure sleep for the duration of the study. The Actiwatch 2 was expected to be worn on the non-dominant wrist for 24 hours each day of the study, and was only removed for showering, swimming, and contact sports. Subjective sleep quality was recorded in the sleep log using the question: “how did you feel when you woke up this morning?”, with a 5-point Likert scale ranging from exhausted (0) to fully rested (4).

Procedures

Day one of the study was marked by a preliminary intake session, based on the availability of the participant and their family/caregiver. During this session, eligibility was verified, and consent was obtained prior to commencing any of the research activities. Additional measures of interest like socioeconomic status were obtained via a self-reported questionnaire (Chaput et al., 2009). The eighth day following intake marked the testing day, whereby participants came back to the laboratory to complete various questionnaires and cognitive tasks. The testing session began at 8:00AM when the participant arrived at CHEO, and the Actiwatch 2® (Philips Respironics, Netherlands) was collected. Given that the CPAT2D study was designed to match the SMART2D study, there were several steps that had to be followed in order to accurately compare participant testing sessions. Those steps, while not relevant to the current study, are found below: following participant arrival, participants were instructed to rest quietly for 5 minutes (and be on their phones, for example) to capture resting anthropometric measurements. Following the rest period, blood pressure, heart rate, blood oxygen saturation, and median arterial pressure were measured, finishing with weight and body composition. Between 8:30AM and 10:30AM, participants completed several sleep quality questionnaires and mood questionnaires based on the previous week.

Around 9:45AM, participants had their heads measured using the 10/20 electroencephalography (EEG) system, to determine which size of the EEG EasyCap would provide the best fit. Each cap included a 32-channel set of electrodes that was then connected to a software program called BrainVison Recorder, which, once calibrated, provided real-time recordings of brain waves.

The EEG system was used to measure electrical activity over the scalp during two cognitive tasks: a dual-task paradigm, and an affective shifting task that used a go/no-go paradigm. After the tasks were completed, an *ad libitum* homogenous test meal was provided to the participants (up to 2800 kcal). At the end of the testing session, participants were issued $25.00 and 10 hours of volunteer community service as compensation for their participation (see Table 1 for full participant testing schedule).

Table 1. *The timing of the events relevant to the CPAT2D study.*

Time	Laboratory Testing Schedule
8:00am	Laboratory arrival time (hand in watch and booklet with the completed logs)
9:45am	Begin electroencephalography capping process
12:45pm	Affective Shifting Task

Note. The CPAT2D schedule was modelled after the testing schedule for the SMART2D study developed by Caroline Dutil. Table 1 represents the laboratory testing sequence of events. SMART2D refers to sleep manipulation in adolescents with type 2 diabetes. Abbreviations: CPAT2D, cognitive performance in adolescents with type 2 diabetes; SMART2D, sleep manipulation in adolescents at risk of type 2 diabetes.

Protocols

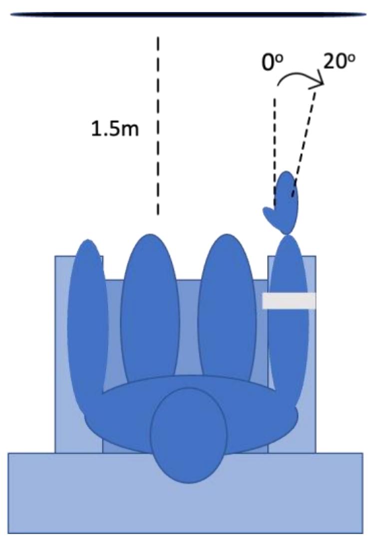
The present study examined one dimension of the CPAT2D study. More specifically, the aim of this project was to compare spectral power during a go/no-go paradigm task between a case group (adolescents with T2D) and controls (participants in the first control group were apparently healthy and those in the second control group were at risk of T2D). The outcome measure for this study was spectral power; more specifically, five main frequencies (theta waves, alpha waves - low and high, and beta waves - low and high) during four conditions of the affective shifting task designed by Muele et al. (2014). These spectral frequencies were more specifically compared during four main conditions: “go” on food images, “go” on object images, “no go” on food images, and “no go” on object images, further partitioned by regions of interest in the brain based on spatial location and success of electrode placement. The task for participants was to react to the "go" stimulus by making a targeted 20-degree wrist extension as quickly and accurately as possible, and to refrain from doing so if a distractor stimulus was presented. This task was composed of one practice block (with ten trials) and sixteen testing blocks (with twenty trials each). In each block there was a 1:1 ratio of “go” stimuli to distractor stimuli, with go stimuli being images of either food (F) or objects (O). In total, there were 320 images of food and objects in this task. The order of block presentation was also pseudo-randomized across participants. Unlike typical go/no-go paradigms, the target go stimulus was alternated every two blocks (i.e., OOFFOOFF…or FFOOFFOO…). The blocks were labeled one to sixteen and were either presented in this order or from sixteen to one (see Figure 2 for block order). Spectral power, including theta, alpha (low and high) and beta (low and high) waves were compared between the three groups during four specified conditions of this task (“go” on food images, “go” on object images, "no-go’s” on images of food, and “no-go’s” on images of objects).

Figure 2. *Block order for the affective shifting task.*

Note. Blocks were either presented one to 16 or starting at the 16th block back to the 1st. This figure was adapted from materials developed by Caroline Dutil for the SMART2D study.

This affective shifting task was administered on a 21-inch computer monitor that is situated at eye-level approximately 1.5 meters away from the participant, who was seated in a custom-built chair (see Figure 3).

Figure 3. *Participant setup.*

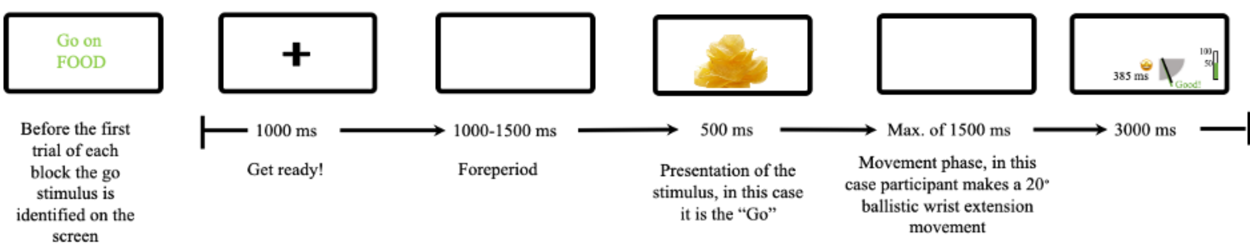


Note. This figure was adapted from materials developed by Caroline Dutil for the SMART2D study.

The chair itself had a backrest, a seat, and an arm rest that positioned the participant's right arm with approximately 20º of shoulder abduction, 90º of elbow flexion, and the right thumb facing upward, forcing the palm of the hand to be internally rotated. The participants wrist lay in a custom-built manipulandum measuring movement kinematics such as velocity, acceleration, and wrist displacement. Two surface electromyography (EMG) electrodes were placed on the right extensor and flexor carpi radialis to measure premotor reaction times. The area of skin under each electrode was exfoliated and cleaned beforehand. Lastly, a reference electrode was placed on the participants right medial epicondyle.

The task was composed of a specific sequence of events, detailed in Figure 4. Visual feedback was given on the computer monitor after each trial based on speed and accuracy. The trial timeline is shown below (see Figure 4).

Figure 4. *Trial timing for a single trial.*



Note. This figure was adapted from materials developed by Caroline Dutil for the SMART2D study. All visual stimuli used in this task were taken from the Food-Pics_Extended image database (Blechert et al., 2014), with the image above corresponding to picture number 0026.

Throughout this task, neural spectral power was being measured using EEG set into Easycap (Easycap GmbH, Germany). Mid distance between the nasion and inion was used as the first landmarking measurement for Cz and head circumference helped to make sure the cap was stretched outward properly. The second landmarking used to find Cz was the midpoint between the two orbicularis oculi bones on either side of the head (located just in front of the ear).

Once the cap had been fitted to the participant's head, the hair beneath each of the 32 electrodes was gently parted using the wooden base of a Q-tip, and the scalp was gently exfoliated using a spot of Abralyt HiCl, high chloride, abrasive electrolyte gel (EasyCap) on the cotton pad of the Q-tip. Electrode impendence was kept below 10 for each of the 32 electrodes in order to preserve the integrity of the signal. The computer used BrainVision Recorder (Brain Products GmbH, Germany) to both show the status of each electrode and the brain waves. The different spectral frequencies were identified and exported using BrainVision Recorder technology. More specifically, individual frequencies were exported by averaging Fast Fourier Transforms of raw EEG data and parsed by range for each of the five waves of interest.

Sleep was measured using a Philips Actiwatch 2®, which is an actigraphy watch capable of measuring several key dimensions of sleep including time in bed, wake after sleep onset, sleep efficiency, exposure to light, and sleep time. The watch was worn on the participant's non-dominant wrist, and sleep data were downloaded onto the computer and verified at the testing session.

Statistical Analysis

Descriptive statistics of the categories of participants are provided as means and standard deviations where relevant. The criterion to reject the null hypothesis was set a priori at an alpha level of 0.05. Mixed model ANOVAs were used to test the effect of group on spectral densities during each of the four trial conditions: "go" trials on food, "go" trials on objects, "no-go" trials on food, and “no-go” trials on objects. Only correct trials were analyzed in order to focus specifically on the differences between “go’s” and “no-go’s” and food versus objects.

For significant main effects, the Least Significant Difference (LSD) post-hoc comparison was used. While this post-hoc method is the least conservative, the mixed model ANOVA had so many comparators that using a more conservative post-hoc test ran the risk of increasing the probability of Type 2 Error.

Theta (4.5-7.5Hz), alpha (low = 8-10Hz, high = 11-13Hz), and beta (low = 14-20Hz, high = 21-30Hz) waves were recorded and cleaned using BrainVision Recorder and Analyzer (Brain Products GmbH, Germany).

During the testing sessions, impedance for some electrodes occasionally exceeded the minimal quality threshold (which was <10 ohms). This created some issues in data quality and for this reason, the specific region of interest chosen in this study was not only based on spatial location, but also the electrodes that consistently had clear readings. As such, the region of interest was comprised of electrodes C3, C4, Fz, Cz and Pz, which were pooled together and averaged into one value (per group, per condition) for the statistical analyses.

All spectral waves were segmented to -500ms before stimulus onset to 500ms after stimulus presentation with an additional baseline correction between -500ms and -400ms (Hume et al., 2015). To test for sphericity, Mauchly’s test was used along with the Greenhouse-Geisser corrected degrees of freedom, where necessary. Partial eta squared values are provided to give an estimate of the effect size. All analyses were completed using IBM ® SPSS version 25 Statistics software.

Chapter 4: Results

Participants

A total of 33 participants were included in this study (17 female, 16 male), with 11 adolescents in the case group and 22 adolescents in the control groups: 11 in control group 1, the apparently healthy controls (AH), and 11 in control group 2, the at-risk of type 2 diabetes controls (at risk of T2D). Three extreme outliers (one per group) were removed due to poor EEG signal. This was a result of high electrode impedance, which would have greatly skewed the data if included. This was determined upon visual inspection of the raw data.

Participants in the case group were matched 1:1:1 to each of the control groups, based on six characteristics: age, school grade, sex and/or gender, sleep duration, socioeconomic status, and pubertal status. Nine out of the 11 case participants were matched on four of the six criteria (to each of the two control groups) and the remaining two case participants were matched on five of the six criteria.

Descriptive statistics for the case and control participants can be found below in **Table 2.** All values are presented as means with standard deviation, where applicable. A one-way ANOVA was run to determine if the case group (T2D) differed significantly from the control groups (AH and at-risk of T2D) on the matching criteria. Adolescents in all three groups were of similar age ($p=.704$), school grade ($p=.857$), and average sleep duration ($p=.908$). Pubertal status also did not differ between the three groups ($p=.417$). The full descriptive statistics of the categorical data (sex, ethnicity, and SES) are detailed in **Table 2**.

Table 2. *Descriptive statistics for the CPAT2D study.*

Characteristic	T2D *n*=11	AH *n*=11	AT-RISK OF T2D *n*=11
Age in years	15.6 (0.9)	15.4 (0.9)	15.3 (1.3)
School Grade	10.2 (1.3)	10.4 (0.9)	10.5 (1.3)
Sex, self-reported *n*%			
Male	36%	54%	54%
Female	67%	46%	46%
Sleep Duration (minutes/night)	436.3 (60.3)	440.9 (29.5)	433.2 (22.5)
Ethnicity, self-identified *n*%			
Non-Caucasian	54%	0%	18%
Caucasian	46%	100%	82%
SES by proxy, *n*%			
Very low to low	45%	18%	18%
Medium	36%	36%	36%
Medium-high to high	18%	45%	45%
Puberty (Tanner score)	4.14 (0.45)	4.10 (0.80)	4.41 (0.49)
Body Mass Index in kg/m^2	34.7 (7.7)	20.7 (2.4)	40.0 (4.1)
Percentile by age	>99th	33rd	>99th

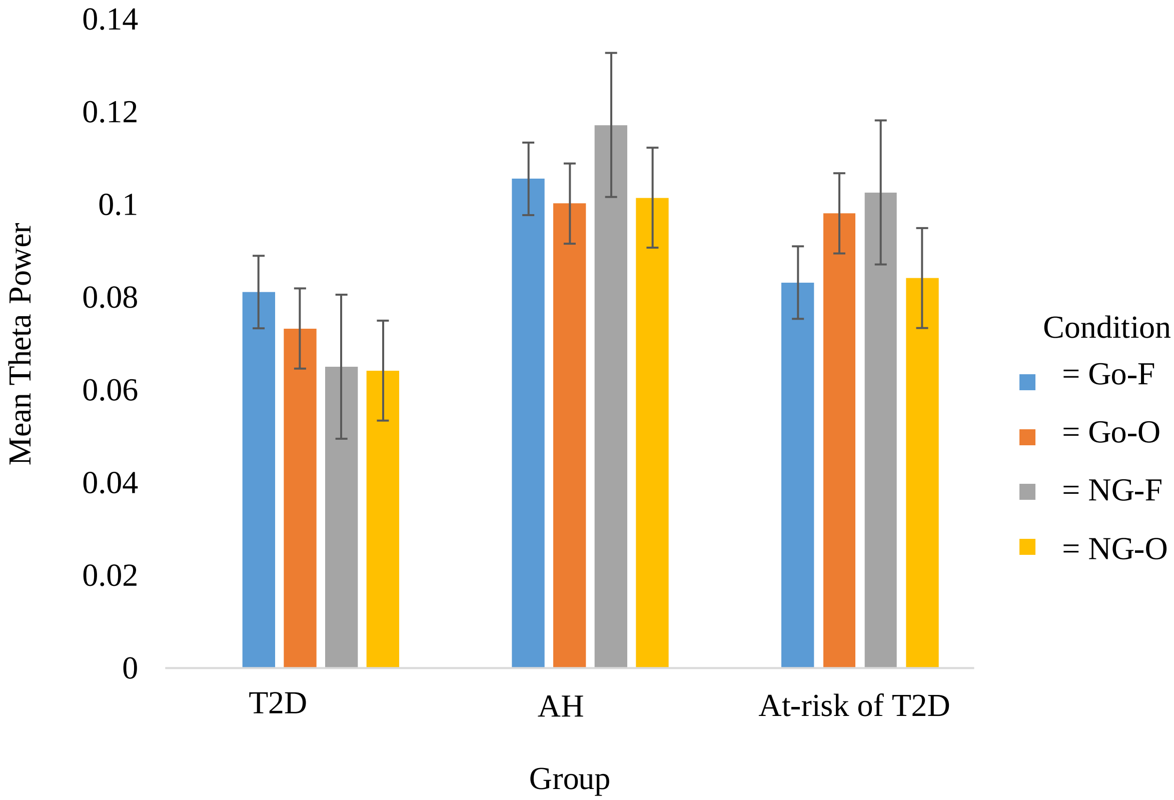
Note. The study includes 11 cases (adolescents with T2D) and 22 controls (11 in group 1 (AH) and 11 in group 2 (at-risk of T2D). Adolescents from the At-Risk group were recruited as part of the Sleep Manipulation in Adolescents with T2D Study (SMART2D). Values are presented as means with standard deviation. *Abbreviations:* CPAT2D refers to cognitive performance in adolescents with type 2 diabetes; T2D refers to type 2 diabetes; AH refers to apparently healthy; and SES refers to socioeconomic status.

Spectral Power

Theta

A mixed model ANOVA was conducted to assess differences between groups and conditions in the theta frequency and using the region of interest (ROI). Three extreme outliers were removed from the data (one per group) in order to correct for extreme values. Mauchley's test of sphericity also indicated that the assumption of sphericity had been violated and therefore homogeneity of variance was corrected using Greenhouse-Geisser corrected degrees of freedom. There was no main effect for group, $F(2,30)=.614$, $p=.548$, $n_2=.039$. There was also no main effect found for condition, corrected by Greenhouse-Geisser $F(2.374, 71.217)=1.229$, $p=.302$, $n_2=.039$. Lastly, there was no interaction between group and condition, corrected by Greenhouse-Geisser $F(4.748, 101.217)=1.326$, $p=.265$, $n_2=.081$ (see Figure 5).

Figure 5. *Mean Theta power at the region of interest for each condition during the affective shifting task between adolescents with type 2 diabetes (cases), adolescents who are apparently healthy (controls 1) and adolescent with obesity but without type 2 diabetes (controls 2).*

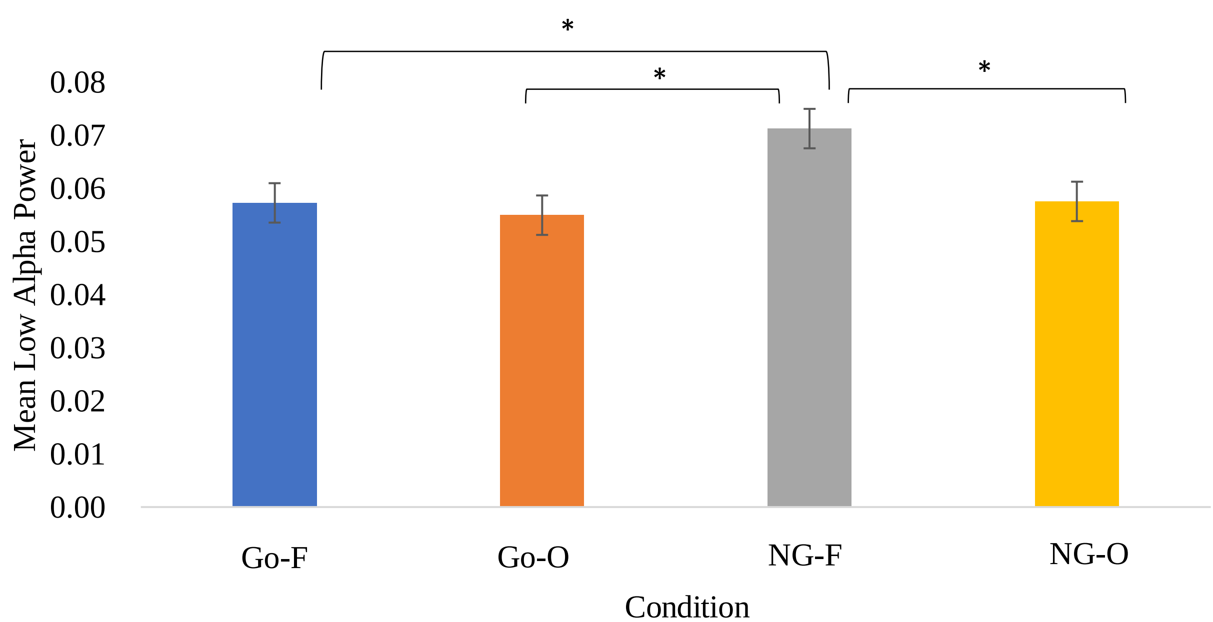


Note. A mixed model ANOVA revealed no significant difference between group (p=.548), no main effect for condition (p=.302), and no interaction, (p=.265). Region of interest include electrodes C3, C4, Fz, Cz, and Pz. Abbreviations: AH, apparently healthy; T2D, type 2 diabetes, Go-F, "go" movement in response to an image of food; Go-O, "go" movement in response to an image of an object; NG-F, not moving in response to an image of food where the target "go" stimulus is an object; NG-O, not moving in response to an image of an object where the target "go" stimulus is a food.

Low Alpha

A mixed model ANOVA was conducted to assess differences between groups and conditions in the low alpha frequency. Three extreme outliers were removed from the data (one per group) in order to correct for extreme values. Mauchley's test of sphericity also indicated that the assumption of sphericity had been violated and therefore homogeneity of variance was corrected using Greenhouse-Geisser corrected degrees of freedom. There was no main effect found for group in the low alpha frequency band, $F(2.30)=.486$, $p=.620$, $n_2=.031$. There was a main effect found for condition, $F(2.129, 63.874)=3.584$, $p=.031$, $n_2=.107$. The Least Significant Differences post-hoc comparisons indicated that average low alpha during the “go” on food images condition was significantly lower (-.014) than low alpha in the “No Go on Food Images” ($p=.042$). It was also found that low alpha during the “Go on Object Images” condition was significantly lower (-.016) than the “No Go on Food Images” condition ($p=.024$). Lastly, mean low alpha power during the “No Go on Food Image” was found to be higher (.014) than the “No Go on Object Image” condition ($p=.029$). There was no interaction found between group and condition, $F(4.258, 93.874)=.546$, $p=.713$, $n_2=.035$. See Figure 6.

Figure 6. *Mean Low Alpha power at the region of interest for each condition during the affective shifting task, collapsed across groups.*

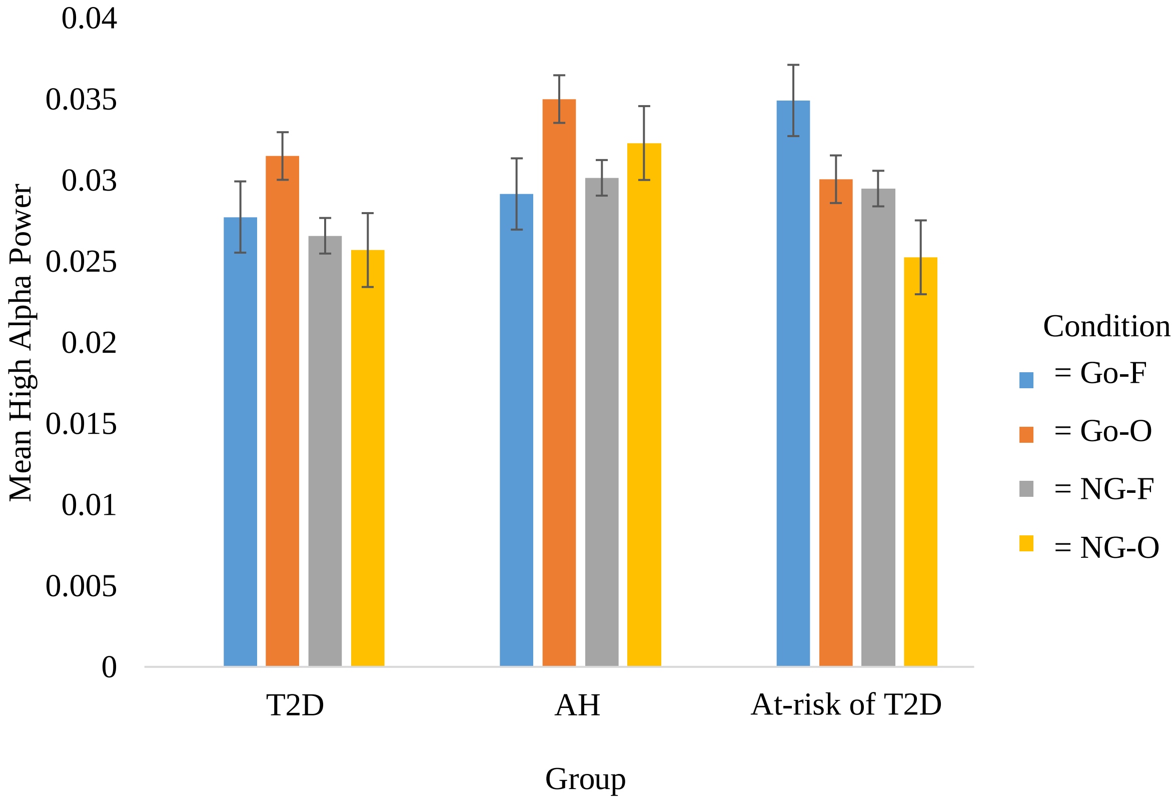


Note. A mixed model ANOVA revealed no significant difference between group ($p=.620$), a main effect for condition ($p=.031$), and no interaction, ($p=.731$). NOTE: * represents a difference when $p<.05$. Region of interest include electrodes C3, C4, Fz, Cz, and Pz. Abbreviations: Go-F, “go” movement in response to an image of food; Go-O, “go” movement in response to an image of an object; NG-F, not moving in response to an image of food where the target “go” stimulus is an object; NG-O, not moving in response to an image of an object where the target “go” stimulus is a food.

High Alpha

A mixed model ANOVA was conducted to assess differences between groups and conditions in the low alpha frequency. Three extreme outliers were removed from the data (one per group) in order to correct for extreme values. Mauchley’s test of sphericity also indicated that the assumption of sphericity had been violated and therefore homogeneity of variance was corrected using Greenhouse-Geisser corrected degrees of freedom. There was no main effect found for group, $F(2,30)=.098$, $p=.907$, $n_2=.006$. There was also no main effect found between conditions, $F(2.450, 73.495)=.722$, $p=.515$, $n_2=.024$. Lastly, there was no interaction found between group and condition, $F(4.900, 103.495)=.532$, $p=.748$, $n_2=.034$. See Figure 7.

Figure 7. *Mean High Alpha power at the region of interest for each condition during the affective shifting task between adolescents with type 2 diabetes (cases), adolescents who are apparently healthy (controls 1) and adolescent with obesity but without type 2 diabetes (controls 2).*

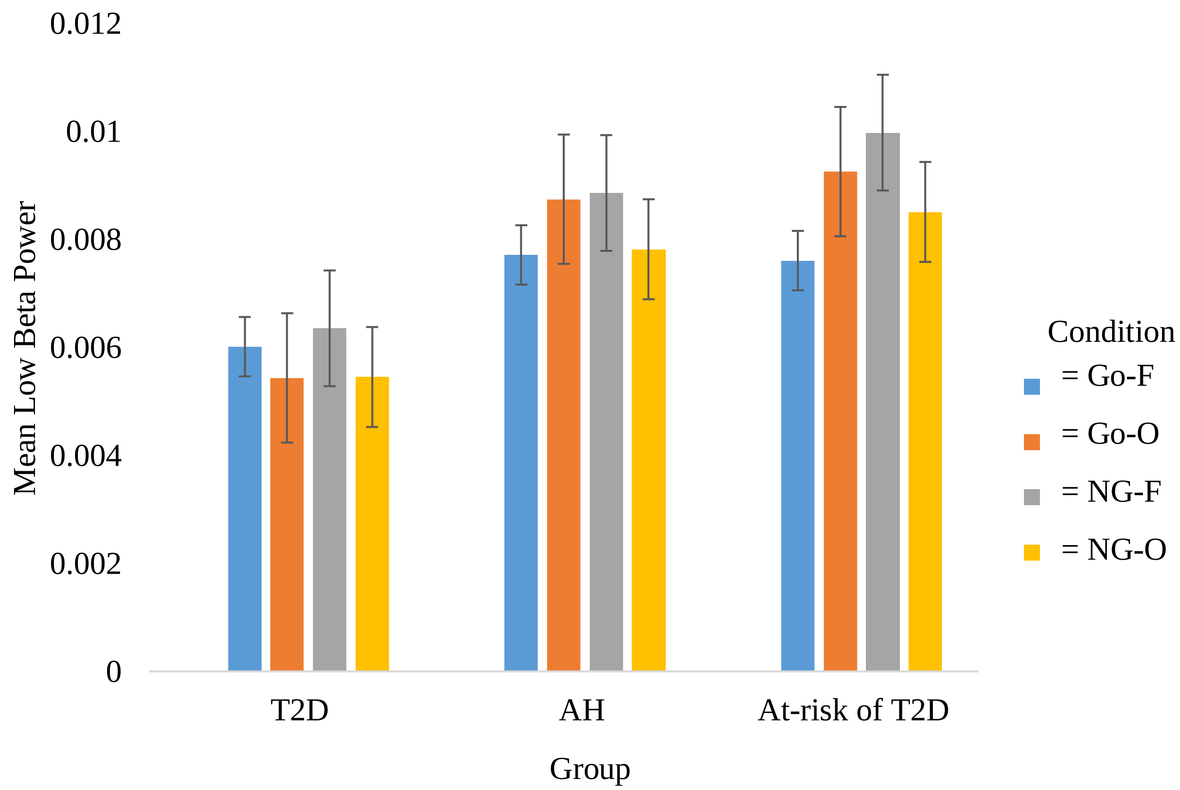


Note. A mixed model ANOVA revealed no significant difference between group (p=.907), no main effect for condition (p=.515), and no interaction, (p=.748). Region of interest include electrodes C3, C4, Fz, Cz, and Pz. Abbreviations: AH, apparently healthy; T2D, type 2 diabetes. Go-F, “go” movement in response to an image of food; Go-O, “go” movement in response to an image of an object; NG-F, not moving in response to an image of food where the target “go” stimulus is an object; NG-O, not moving in response to an image of an object where the target “go” stimulus is a food.

Low Beta

A mixed model ANOVA was conducted to assess differences between groups and conditions in the low beta frequency. Three extreme outliers were removed from the data (one per group) in order to correct for extreme values. Mauchley’s test of sphericity also indicated that the assumption of sphericity had been violated and therefore homogeneity of variance was corrected using Greenhouse-Geisser corrected degrees of freedom. There was no main effect for group in the low beta band, $F(2,30)=.988$, p=.384, $n_2=.062$. There was also no main effect found for condition, $F(2.662, 79.867)=2.424$, p=.071, $n_2=.075$. Lastly, there was no interaction found between group and condition, $F(5.324, 109.867)=.672$, p=.655, $n_2=.043$. See Figure 8.

Figure 8. *Mean Low Beta power at the region of interest for each condition during the affective shifting task between adolescents with type 2 diabetes (cases), adolescents who are apparently healthy (controls 1) and adolescent with obesity but without type 2 diabetes (controls 2).*

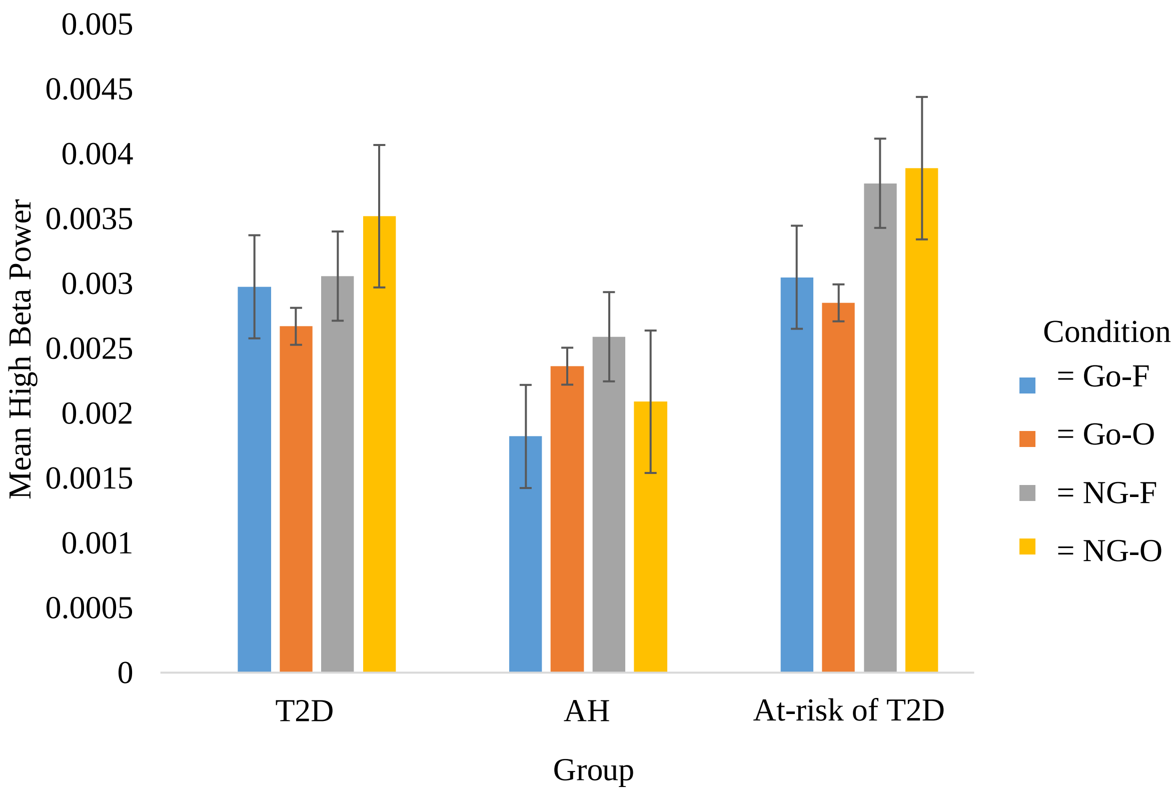


Note. A mixed model ANOVA revealed no significant difference between group (p=.384), no main effect for condition (p=.071), and no interaction, (p=.655). Region of interest include electrodes C3, C4, Fz, Cz, and Pz. Abbreviations: AH, apparently healthy; T2D, type 2 diabetes, Go-F, “go” movement in response to an image of food; Go-O, “go” movement in response to an image of an object; NG-F, not moving in response to an image of food where the target “go” stimulus is an object; NG-O, not moving in response to an image of an object where the target “go” stimulus is a food.

High Beta

A mixed model ANOVA was conducted to assess differences between groups and conditions in the high beta frequency. Three extreme outliers were removed from the data (one per group) in order to correct for extreme values. Mauchley's test of sphericity also indicated that the assumption of sphericity had been violated and therefore homogeneity of variance was corrected using Greenhouse-Geisser corrected degrees of freedom. First, there was no main effect found between groups, $F(2,30)=1.314$, $p=.284$, $n_2=.081$. There was also no main effect found for condition, $F(2.062, 61.847)=2.482$, $p=.090$, $n_2=.076$. Lastly, there was no two way interaction found between group and condition, $F(4.123, 91.847)=.962$, $p=.437$, $n_2=.060$. See Figure 9.

Figure 9. *Mean High Beta power at the region of interest for each condition during the affective shifting task between adolescents with type 2 diabetes (cases), adolescents who are apparently healthy (controls 1) and adolescent with obesity but without type 2 diabetes (controls 2).*



A mixed model ANOVA revealed no significant difference between group (p=.284), no main effect for condition (p=.090), and no interaction, (p=.437). Region of interest include electrodes C3, C4, Fz, Cz, and Pz. Abbreviations: AH, apparently healthy; T2D, type 2 diabetes, Go-F, "go" movement in response to an image of food; Go-O, "go" movement in response to an image of an object; NG-F, not moving in response to an image of food where the target "go" stimulus is an object; NG-O, not moving in response to an image of an object where the target "go" stimulus is a food.

Chapter 5: Discussion

Overview

This thesis was a part of a larger study that occurred at CHEO entitled CPAT2D. The overarching goal of this project was to examine cognitive performance in adolescents with type 2 diabetes (T2D). To our knowledge, this study was the first of its kind comparing spectral power in adolescents with T2D to those with obesity and those who are apparently healthy.

The purpose of the specific experiment described above was to determine if there were spectral power differences in adolescents with T2D compared to those who were at risk of T2D and those who were apparently healthy during an affective shifting task, using a case-control study design. The specific research question that this project aimed to answer was whether adolescents with T2D would demonstrate differences in spectral power during an affective shifting task (measuring both behavioural inhibition and attention) as compared to adolescents with obesity and adolescents who are apparently healthy. More specifically, the frequencies being compared were theta waves (4.5-7Hz), low alpha waves (8-10Hz), high alpha waves (11-13Hz), low beta waves (14-20Hz), and high beta waves (21-30Hz).

Adolescents with T2D were matched 1:1:1 with adolescents at risk of T2D and adolescents who were apparently healthy. The three groups were matched on school grade, sex and/or gender, pubertal status, socioeconomic status, and sleep duration. Cognition was measured with an affective shifting task that used a go/no-go paradigm, pairing an emotionally salient stimulus (food) with a neutral stimulus (objects). Cognitive performance was examined throughout the task by looking at spectral power in five main frequency bands: theta, low alpha, high alpha, low beta, and high beta. In order to avoid washing out any effects, spectral power was more specifically examined in five main electrodes (C3, C4, Fz, Cz, and Pz), which were carefully chosen due to their location and proximity to key regions of the brain activated during an affective shifting task. Lastly, these frequencies were more specifically compared between the three groups of adolescents during four main conditions: “go” trials during food images, “go” trials during object images, “no-go” trials during food images, and “no-go” trials during object images (all conditions without error).

There were four main hypotheses for this project: Firstly, it was thought that adolescents with T2D would have lower overall spectral power densities in all frequency bands than the control group (adolescents with obesity and apparently healthy) (Hyllienmark et al., 2005, Eeg-Olofson and Peterson, 1966). Secondly, it was thought that given the higher demand on inhibitory processing required for no-go stimuli, spectral power, specifically alpha and theta, would be higher during no-go trials compared to go trials (Funderud et al., 2012 & DeLaRosa et al., 2020, & Kuo et al., 2022). Thirdly, given the higher demand on attention required on no-go trials, it was hypothesized that spectral power, specifically alpha, would be higher for all groups on no-go trials compared to go trials. Lastly, in terms of saliency of the stimulus, it was hypothesized that the spectral power would be higher for food (more salient stimulus) compared to objects (neutral stimulus) due to the increased demand on inhibitory control (Nijs et al., 2008, & Gallardo-Moreno et al., 2020, & Imperatori et al., 2015).

This study was the first of its kind and provided much insight into spectral power in adolescents with T2D compared to those without. This discussion will compare the findings of this study to the recent and existing literature on the topic. Firstly, group type will be discussed, followed by each spectral frequency in each of the four conditions. Possible reasonings for the findings will be discussed next, followed by strengths, limitations, potential significance, and a brief conclusion.

Theta Waves

As seen in Figure 5, there was no difference in group type or condition. Given the mixed results in the literature surrounding theta wave differences in go versus no-go's, the lack of significance in theta waves between the go and no-go trials was not surprising. Brier and colleagues (2010) also did not find significance in theta waves between go and no-go trials, which is further supported by findings from this study.

In terms of group type, it was hypothesized that all spectral frequencies would differ between adolescents with T2D and the control groups. As mentioned in the review of the literature, Eeg-Olofsson and Petersen (1966) found that children with diabetes (unspecified type) had increased activity in the theta band compared to those without. There were, however, no differences found between the three groups of adolescents. While this was initially surprising, it is not necessarily unexpected, given that much of the literature surrounding spectral power and diabetes focused on either adults with T2D, adolescents with T1D, or focused exclusively on resting state brain waves (Benwell et al., 2020, & Hyllienmark et al., 2005).

Additionally, as mentioned in the review of the literature, the role of theta waves shifts between childhood and adulthood, with theta waves indicating drowsiness in childhood and working memory in adulthood (Amin & Malik, 2013). Given that the affective shifting task required a heavier cognitive load, it is also possible that theta waves were all similar because of the higher demand on the brain in all groups.

Alpha Waves (low and high)

As seen in *Figures 6* and *7* of this study, there was a significant effect for condition. More specifically, low alpha waves were higher during the “no-go” on food image condition compared to all three others. This finding directly aligns with the findings from Funderud et al. (2012) and Kuo et al. (2022) which was hypothesized to be due to the higher cognitive demand required to inhibit a “go” response.

There was no main effect found for group in the alpha frequency. Given the existing literature, it was hypothesized that group differences would be found, especially considering that there are differences in adults with T2D (Benwell et al., 2020) and children with T1D (Hyllienmark et al., 2005). Given that this study was the first to compare spectral power in three groups of adolescents, more specifically, adolescents with T2D, there was not much of a basis of literature to compare. The goal was to provide exploratory findings to the scientific community and to deepen the understanding of T2D’s impact on the developing brain, particularly as it pertains to spectral power. One possible mechanism for this finding is that perhaps the spectral power differences associated with T2D in adulthood do not appear until later in disease progression, and therefore the disease has a lesser impact on the brain during adolescence. Ultimately, this is a positive finding for adolescents with T2D, as it possibly suggests that their brains are producing similar densities of low alpha waves compared to their apparently healthy and with obesity counterparts.

As seen in Figure 6, there was a significant difference in condition for the low alpha frequency waves. As seen in the post-hoc analyses, low alpha waves were higher in the “no-go” on food images compared to the “go” on food images, the “go” on object images, and the “no-go” on object images. This discovery directly aligns with findings from Funderud et al. (2012)

and Kuo et al. (2022), who both found an increase in alpha waves during “no-go” conditions, attributing the result to higher cognitive demand. Additionally, alpha was also higher during the food-oriented no-go trials, which makes sense considering the link between emotionally salient stimuli and spectral power. Researchers have found that salient stimuli (i.e. food) elicit higher spectral power than neutral stimuli, which supports the finding that low alpha is higher during the “no-go’s” for food compared to the “no-go’s” for objects (Luo et al., 2009, & Nijs et al., 2008, & Funderud et al., 2012).

Beta Waves (low and high)

Given the wide range of spectral power in the beta frequency, the analyses were run separately; however, due to the lack of significant findings, they will be discussed together. As seen in Figure 8 and 9, there were no main effects for condition, electrode, or group type in the beta frequencies. Firstly, there was no main effect found for group, which was not necessarily surprising, for several reasons. The literature has shown that excess weight impacts beta power; however, the finding from Hume and colleagues (2015) did not involve a complex cognitive task, therefore it is possible that tasks requiring higher cognitive demand do not yield group differences. Hume and colleagues’ study (2015) also only examined adults and not adolescents and therefore it was not clear if there would be group differences in the beta frequency between these populations.

Additionally, as mentioned in the review of the literature, Hyllienmark and colleagues (2005) found that youth with T1D had lower beta power compared to those without, which was part of the foundation for the hypothesis that adolescents with T2D would have lower beta power. Given the lack of literature surrounding beta waves and T2D in adolescents, the findings from this study are also somewhat expected and offer valuable contributions to the knowledge gaps. Given that these group differences were not found in adolescents with T2D, it might suggest that the adolescent brain is still producing beta waves at a comparable rate to otherwise healthy adolescents. Secondly, while Benwell and colleagues (2020) found group differences in some frequencies (comparing adults with T2D to adults without), they did not find any group differences in the beta band during their complex cognitive task. Therefore, it was somewhat expected that there would not be group differences in adolescents with T2D compared to those without.

The main outcomes of this study were examining spectral power during each of the four conditions, for which there was much literature supporting spectral differences between go and no-go trials. The lack of significant difference in the beta band between conditions was therefore surprising because Cooper and colleagues (2016) found that beta waves increased during motor preparation, for which the task used in this study required significant motor preparation. More specifically, given that correct "go" trials were compared to correct "no-go" trials, it would have made sense to find higher beta power during the go trials, however this was not the case. As mentioned in the review of the literature, Cooper et al.'s study (2016) looked specifically at healthy adults and therefore research comparing adolescents, especially special populations such as those with T2D, is still preliminary and therefore not finding significant differences between conditions was not altogether unsurprising either.

The findings by condition for this study were more in line with results from the study by Ergen et al (2014). Their findings revealed no significant differences in beta power between congruent and incongruent trials in a Stroop task. These conditions are comparable to the go and no-gos of an affective shifting task, as they are both complex cognitive tasks. As mentioned in the literature, findings were inconsistent between gos and no-gos, and therefore any result would have aligned with what has previously been found.

Possible Mechanisms

While it was somewhat unexpected that there were no group differences found in any of the five frequencies, there are several possible reasons for this. First, as detailed in the review of the literature, adolescence is marked by a period of rapid growth, both physically and cognitively. Larsen & Luna (2018) emphasized how much the prefrontal cortex (responsible for functions like working memory and inhibition) develops during adolescence. It was also established in the literature that the affective shifting task heavily challenges both working memory and inhibition (Muele et al., 2014). It is possible that all groups had similar spectral power because all three groups were undergoing rapid changes to the brain and were therefore performing in similar ways. It was also key that one of the inclusion criteria for adolescents with T2D was that they had to be adhering to a T2D medication regime. This is important to note because had there been significant group differences in spectral power, they may not have been attributable to metabolic factors alone. This area would greatly warrant future research.

It is important to note that there has never been a study of this kind before and therefore the results were primarily exploratory. However, it is also possible that the success of the matching between groups provided an accurate comparison and that in this demographic of adolescents, there are no significant differences in spectral power.

Limitations

While a case-control study is a good study design, this study had several limitations. First, it had a relatively small sample size per group, which was due to a variety of factors. This had a major impact on the power of the study. As seen in the Figures, the results did seem to be trending towards significance, more specifically between groups, however the sample sizes were quite small and therefore the potential differences did not surpass the threshold. In addition, the initial calculation for power used for this study was based on reaction time and not spectral power. It is therefore also possible that the minimum sample size would have been different, had the calculation been done based on spectral power.

Namely, the global pandemic of COVID-19 drastically impacted recruitment, more specifically for adolescents with T2D. Given that one of the primary proposed methods of recruitment was in-person through the CHEO endocrinology clinic, the restrictions in the hospital made it difficult to contact and recruit the desired number of participants. As mentioned above, three participants also had to be removed, as they were extreme outliers, further reducing the sample size. This greatly impacted the power of the study, as there were only 36 participants (12 in the case and 24 in the controls) to begin with.

The issue of power further affected the ability to determine if there were any underlying mechanisms responsible for group differences. For example, Yau and colleagues (2010) noted in their article that the structural brain differences were not attributable to education or socio-economic status, which are factors which have been linked with T2D (Hill-Briggs et al., 2020). Instead, Yau et al. (2010) proposed that these changes in the brain were due to metabolic differences and vascular changes. Unfortunately, due to the lack of statistical power in this study, meaningful conclusions surrounding possible mechanisms could not be drawn. Future research with a larger sample size is warranted.

Another limitation of the study was that while participants were matched 1:1:1 between the three groups, there were two adolescents in the T2D group who had extreme sleep scores. One had stayed up all night for two of the seven nights during which sleep duration was measured and the other was suffering from depression and had a significantly longer sleep duration. This made it harder to match those two on sleep duration, which was an important confounding variable, given how much sleep impacts the brain.

A third limitation of this study was that on occasion, the signal of certain electrodes was lost. This did also impact the ability to select a region of interest. The most frontal electrodes often experienced the most impedance, which impacted the signal. For this reason, the frontal electrodes were not chosen for the region of interest, which was limiting because many of the cognitive domains challenged by the affective shifting task were frontal-lobe dominant, such as behavioural inhibition and working memory (Funahashi, 2017). While this was rare and was caught within one or two trials of the task, it was nonetheless an issue, as it also pulled the signal of other electrodes. Luckily, those trials were able to be removed before analysis.

Strengths

A major strength of this study was the study design. Using a case-control study, matching adolescents between groups on so many factors created three, highly comparable groups, which allowed for meaningful conclusions to be drawn. Second, the fact that this study was the first of its kind to compare spectral power between these three groups during a complex cognitive task offered invaluable information to the scientific community. Additionally, the study design was based on the SMART2D study, which had already been active for over a year by the time this study began. This is important to consider because any issues with the protocol were addressed prior to starting the CPAT2D study, resulting in a strong design and a smooth testing day for participants.

An additional strength of this study is that the affective shifting task used in this study was a new instrument, developed specifically for the SMART2D study. Despite the novelty, the differences found in the low alpha band (by condition and stimulus type) were consistent with the literature findings with similar tasks. This suggests that the affective shifting task used in this study is a strong measure of complex cognition.

Another strength of this study was that there were many other variables also measured, including subjective sleep quality, screentime and sedentary behaviours, food preferences, and mental health questionnaires, which allows for meaningful comparisons to be drawn between other variables or co-variables in future research. Additionally, given that three groups of adolescents were compared, it allowed us to see the progression (or lack thereof) of the disease on the spectral power in the developing brain.

Significance

These findings are of high significance to the scientific community because this study was the first of its kind comparing spectral power densities in adolescents with T2D compared to those without. Although preliminary and exploratory, these findings suggest that adolescents with T2D are not performing differently on cognitive tasks in terms of spectral power, compared to apparently healthy adolescents or adolescents with obesity. These findings also offer valuable insight into the timeline of disease on spectral power, meaning that research findings have shown that adults with T2D have lower spectral power, but this study indicates that perhaps those changes do not develop until later. Altogether, the most significant part of this study was that it provided valuable and novel insight into spectral power in different populations (in key regions of the brain) during a cognitive task, offering new information to the scientific community and contributing to the literature.

Conclusion

As mentioned, this study was the first of its kind examining potential spectral power differences in adolescents with T2D compared to those without during a complex cognitive task. This study used a case-control design, matching adolescents with T2D to those at risk and those who were apparently healthy 1:1:1. Preliminary findings suggest that there are no group differences in theta, low alpha, high alpha, low beta, or high beta frequencies, suggesting that the brains of adolescents with T2D are producing spectral power at comparable frequencies to healthy brains (and those with obesity). Given that adults with T2D have lower frequencies, future research could compare young adults with T2D to those without, to better understand when the group differences begin to appear.

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Appendices

Appendix A: CHEO REB Letter of Approval

From: nanderson@cheo.on.ca <nanderson@cheo.on.ca>
Sent: Thursday, October 8, 2020 9:48 AM
To: Chaput, Jean-Philippe
Cc: Anderson, Natalie
Subject: REB Protocol No 20/102X - Final Approval - Delegated Review

EXTERNAL MAIL*

CHEO

CHEO Research Ethics Board
Approval - Delegated Review

REB Protocol No: 20/102X
ROMEO File No: 20200433
Principal Investigator: Dr. Jean-Philippe Chaput
Project Title: CHEOREB# 20/102X - Cognitive Performance in Adolescents with Type 2 Diabetes (CPAT2D) study: Association between sleep duration and cognitive performance in adolescents with type 2 diabetes and those without

Protocol Status: Active
Approval Date*: October 08, 2020
Approval Expiry Date:** September 15, 2021

Documents Reviewed & Approved:

Document Name	Comments	Version Date
Recruitment Materials	Recruitment Poster	2020/09/09
Recruitment Materials	Recruitment Social Media Version 2	2020/10/05
Other Document	Appendix A_CPAT2D_Safety Protocol	2020/10/05
Protocol	Protocol_CPAT2D	2020/10/05
Assent Form	CPAT2D Study Assent Form	2020/10/05
Consent Form	CPAT2D_Informed Consent	2020/10/05

Documents Reviewed & Acknowledged:

Document Name	Comments	Version Date
Other Document	OCDSB Community Involvement Form - CPAT2D	2020/10/08
Other Document	OCSB Community Involvement Activity Record Form_CPAT2D	2020/10/08
Questionnaire/Survey	Questionnaire - Strength & Difficulties (SDQ)	2020/09/09
Questionnaire/Survey	Questionnaire - Pittsburgh Sleep Quality Index (PSQI)	2020/09/09
Questionnaire/Survey	Questionnaire - Severity Measure for Depression (Child age 11-17)	2020/09/09
Questionnaire/Survey	Questionnaire - Pediatric Quality of Life (PedsQL)	2020/09/09
Questionnaire/Survey	Questionnaire - Positive And Negative Affect Schedule (PANAS)	2020/09/09
Questionnaire/Survey	Questionnaire - Epworth Sleepiness Scale	2020/09/09
Questionnaire/Survey	CPAT2D_FoodScreeningAST Version 2	2020/10/05
Questionnaire/Survey	CPAT2D_PubertalQuestionnaire_MALE Version 2	2020/10/05
Questionnaire/Survey	CPAT2D_PubertalQuestionnaire_Female Version 2	2020/10/05
Questionnaire/Survey	CPAT2D_Screen Time and Sedentary Behaviour Version 2	2020/10/05
Questionnaire/Survey	CPAT2D_Sleep Log Version 2	2020/10/05
Questionnaire/Survey	CPAT2D_ThreeFactorEating Version 2	2020/10/05
Questionnaire/Survey	CPAT2D_VAS Version 2	2020/10/05

This is to notify you that the Children's Hospital of Eastern Ontario Research Ethics Board has granted approval to the above named research study on the date noted above. Your project was reviewed within the delegated stream, which is reserved for projects that involve no more than minimal risk to human participants.

Final approval is granted for the above noted study, with the understanding that the investigator agrees to comply with the following requirements:

1. The investigator must conduct the study in compliance with the protocol and any additional conditions set out by the Board.
2. The investigator is responsible for complying with all applicable guidelines and regulations regarding the ethical conduct of research with humans, as applicable to the research project.
3. Investigators must obtain annual renewal approval prior to the expiry date stated above.
4. The investigator must not implement any deviation from, or changes to, the protocol without the approval of the REB except where necessary to eliminate an immediate hazard to the research subject, or when the change involves only logistical or administrative aspects of the study (e.g., change of telephone number or research staff). As soon as possible, however, the implemented deviation or change, the reasons for it and, if appropriate, the proposed protocol amendment(s) should be submitted to the Board for review and approval.
5. The investigator must, prior to use, obtain approval from the Board for changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials.
6. Investigators must obtain approval from the Board of French version(s) of the consent/assent form(s), unless a waiver has been granted. An interpreter should be offered to participants as required or at the request of the participant throughout the course of research.
7. For clinical drug or device trials, investigators must promptly report to the REB all adverse events that are both serious and unexpected (SAEs) or unexpected and untoward occurrences (including the loss or theft of study data and other such privacy breaches.
8. For SAE reports on clinical drug trials, the investigator must also comply with the hospital-wide Policy regarding, Procedures for Considering Medical Error in the Differential Diagnosis of Severe Adverse Events (SAE) Associated with the Drugs Administered in a Clinical Trial.
9. Investigators must promptly report to the REB any new information regarding the safety of research subjects (e.g., changes to the product monograph or investigator's brochure of drug trials). Where available, any reports produced by the Data Safety Monitoring Board should also be promptly submitted to the REB for acknowledgement.
10. Investigators must notify the REB of any change in study status (closed to accrual, temporary, premature or permanent).
11. Investigators must submit a study closure event form at the conclusion of the study.

If you have any questions, pertaining to this letter, please contact the Research Ethics Board Office.

Regards,

Cécile Bensimon, MA, PhD
Chair, Research Ethics Board
Président, Comité d'éthique de la recherche

***** The final approval date for initial delegated study applications approved with or without modifications will be the date the REB has determined that the conditions of approval have been satisfied.

****** The expiry date of REB approval for initial study applications will be as follows:
• If the date of approval was **on or before** the 15th of the month, the expiry date will be the 15th of the month prior to the date of review and approval by the Chair and/or delegate *in the following year*;
• If the date of review and approval was **after** the 15th of the month, the expiry date will be the 15th of the month in which the date of review and approval by the REB *in the following year*.

Appendix B: University of Ottawa Letter of Approval

03/08/2021

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Lettre d'approbation administrative I Letter of administrative approval

Numéro de dossier / Ethics File Number	H-07-21-7183
Titre du projet / Project Title	Cognitive Performance in Adolescents with Type 2 Diabetes (CPAT2D): Association Between Sleep Duration and Cognitive Performance in Adolescents with Type 2 Diabetes and Those Without(CHEO REB#20/102X)
Type de projet / Project Type	Autre / Other
CÉR primaire / Primary REB	CHEO / CHEO
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	03/08/2021
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	15/09/2021

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Caroline DUTIL	École des sciences de l'activité physique / School of Human Kinetics	Chercheur Principal / Principal Investigator
Jean-Philippe CHAPUT	Département de pédiatrie / Department of Pediatrics	Chercheur principal - site d'examen primaire / Primary review site PI
Anthony CARLSEN	École des sciences de l'activité physique / School of Human Kinetics	Co-superviseur / Co-supervisor
Stuart FOGEL	École de psychologie / School of Psychology	Collaborateur / Collaborator
Gary GOLDFIELD	Département de pédiatrie / Department of Pediatrics	Collaborateur / Collaborator
Irina PODINIC	Département d'épidémiologie et santé publique / Department of Epidemiology and Public Health	Étudiant-chercheur / Student-researcher
Amelia EATON	École des sciences de l'activité physique / School of Human Kinetics	Étudiant-chercheur / Student-researcher

Conditions spéciales ou commentaires / Special conditions or comments:

CHEO REB Protocol No: 20/102X (PI: J.-P. Chaput)

550, rue Cumberland, pièce 154 Ottawa (Ontario) K1N 6N5 Canada
550 Cumberland Street, Room 154 Ottawa, Ontario K1N 6N5 Canada

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www.recherche.uottawa.ca/deontologie I www.recherche.uottawa.ca/ethics

03/08/2021

Université d'Ottawa — Bureau d'éthique et d'intégrité de la recherche

University of Ottawa — Office of Research Ethics and Integrity

L'Université d'Ottawa a signé une Entente, conforme aux exigences de la plus récente version de l'EPTC et tout autre règlement ou législation applicable, permettant au CÉR ci-haut nommé d'être désigné comme CÉR primaire pour les projets de recherche où

1) les activités principales de recherche sont menées sous l'autorité ou sous les auspices de l'établissement lié au CÉR primaire et

2) Une partie du projet est également réalisé sous l'autorité ou sous les auspices de l'Université d'Ottawa.

Cette lettre confirme que l'Université d'Ottawa a autorisé que le CÉR primaire soit le CÉR officiel pour l'évaluation et la supervision de ce projet de recherche. Ceci n'est pas une approbation éthique.

Afin de nous aider à garder votre dossier à jour, veuillez soumettre une copie de toutes demandes de modification, renouvellement d'approbation éthique etc. soumis à et approuvé par le CÉR primaire dès qu'elles sont disponibles.

Cette approbation administrative est valide pour la durée indiquée ci-haut et est sujette aux conditions énumérées dans la section intitulée « Conditions spéciales ou commentaires ».

The University of Ottawa has signed an Agreement, compliant with current TCPS guidelines and any other applicable guidelines or legislation regarding multisite review, allowing the REB named above to serve as Board of Record (BoR) for research projects where

1) the main research activities are conducted within the auspices or jurisdiction of the BoR's institution and

2) parts of the project are also conducted under the jurisdiction or auspices of the University of Ottawa.

This letter confirms that the University of Ottawa has authorized the REB named above to serve as Board of Record for the review and oversight of this research project. This is not an REB approval.

In order to help us keep your file up to date, please submit a copy of all amendment requests, project renewals or any other changes submitted to and approved by the BoR, as they become available.

Administrative approval is valid for the period indicated above and is subject to the conditions listed in the section entitled «Special conditions or comments».

Catherine PAQUET
Directeur / Director
Pour/For **Daniel LAGAREC** Président(e) du/ Chair of the **Comité d'éthique de la recherche en sciences de la santé et sciences / Health Sciences and Sciences Research Ethics Board**

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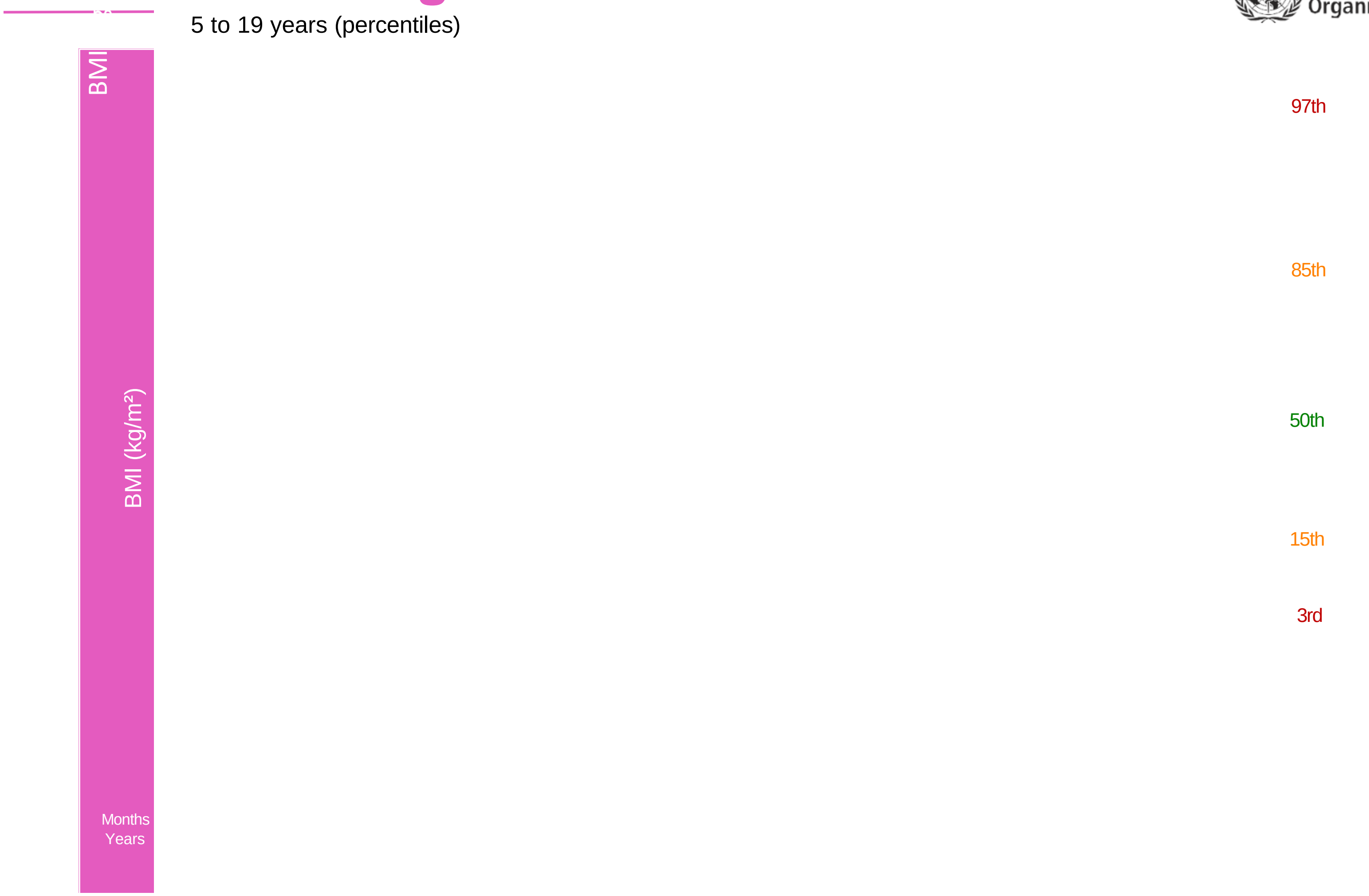
Appendix C: Body Mass Index Growth Charts (World Health Organization)

BMI-for-age GIRLS

5 to 19 years (percentiles)

World Health Organization



2007 WHO Reference

Appendix D: Body Mass Index Growth Charts (World Health Organization)

BMI-for-age BOYS

5 to 19 years (percentiles)

World Health Organization

2007 WHO Reference