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Hippocampus network in response to visual food cues in children and adults: Influence of maternal metabolism and central insulin action

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1. Introduction

1.1 Obesity and Brain Health

Obesity is a significant global health concern, affecting approximately one in eight people worldwide as of 2022 (WHO, 2024). This condition significantly elevates the likelihood of developing insulin resistance, type 2 diabetes (T2D), cardiovascular diseases, cancers, and neurodegenerative disorders, including Alzheimer's disease (Anstey *et al.*, 2011; García-García *et al.*, 2022). Moreover, obesity during pregnancy significantly increases the probability of gestational diabetes mellitus (GDM) (Chu *et al.*, 2007; Yen *et al.*, 2019), which, in turn, contributes to the risk of obesity in the offspring (Kawasaki *et al.*, 2018).

As a complex metabolic condition, obesity has systemic effects, including on the brain. Research has linked it to changes in both brain structure and function, affecting individuals from childhood to old age (Morys *et al.*, 2024). A recent review showed that adults with obesity exhibit alterations in brain morphology, such as reductions in cortical thickness and volume, as well as altered neural activity that negatively affects cognitive function (for review, see (Li *et al.*, 2023a)). These structural and functional alterations are especially pronounced in brain regions within the mesocorticolimbic circuitry, including the striatum, hippocampus, amygdala, insula, and prefrontal cortex (Li *et al.*, 2023a).

The effects of obesity during childhood and adolescence are particularly concerning due to its both immediate and long-term health outcomes. Long-term consequences include a heightened risk of adult obesity and metabolic comorbidities such as insulin resistance (Marcus *et al.*, 2022). Importantly, childhood and adolescence represent crucial stages of brain development, marked by heightened neuroplasticity (Tooley *et al.*, 2021). Both structural and functional studies suggest that reward processing regions mature earlier, whereas the cognitive control system develops later (Konrad *et al.*, 2013; Herting

and Sowell, 2017). This developmental imbalance may contribute to their preference for immediate rewards (e.g., high-caloric foods) over long-term health outcomes, making them vulnerable to obesity (Walker *et al.*, 2017).

Notably, obesity is associated with insulin resistance, a condition in which insulin-sensitive tissues exhibit impaired responsiveness to insulin, resulting from hyperinsulinemia (Barazzoni *et al.*, 2018). Peripheral tissue insulin resistance is a key feature of T2D and GDM. Historically, the brain was thought to be insulin-independent because the central nervous system can utilize glucose without insulin. However, findings in animals and more recently humans show that central insulin resistance has strong metabolic and behavior effects, influencing weight maintenance and the development of T2D and GDM (Kullmann *et al.*, 2020a).

1.2 Central Insulin Signaling and Metabolic Regulation

1.2.1 Brain as an Insulin-sensitive Organ

Although the brain was historically regarded as insensitive to insulin (Hasselbalch *et al.*, 1999; Seaquist *et al.*, 2001)—this view was challenged by the discovery of insulin receptors across various species (Kleinridders *et al.*, 2014). These receptors were widely distributed in regions such as the olfactory bulb, cortex, and subcortical areas. Similarly, postmortem studies in humans revealed widespread insulin receptor expression across the brain (Kullmann *et al.*, 2020a).

Steven Woods and colleagues discovered in the late 1970's, the pivotal role of central insulin in regulating food intake and body weight using intracerebroventricular infusions in baboons (Woods *et al.*, 1979). This discovery fundamentally changed the understanding of insulin's role, indicating that it has important central functions beyond its well-known role in peripheral glucose regulation.

Peripheral insulin can pass through the blood-brain barrier (BBB) via a receptor-mediated, saturable transport process, allowing it to influence various neuronal

functions (Woods *et al.*, 2003; Gray *et al.*, 2014). Once in the central nervous system, insulin is vital for regulating both cognitive and metabolic processes. In particular, it is known that insulin influences memory, appetite, mood regulation, olfactory perception, and peripheral glucose homeostasis (For reviews, see (Kullmann *et al.*, 2020a; Hallschmid, 2021)).

However, central insulin action is thought to be impaired in obesity and T2D (Tabassum *et al.*, 2024). In animals, selectively disrupting insulin receptors in the brain caused overfeeding and obesity (Brüning *et al.*, 2000; Obici *et al.*, 2002; Wardelmann *et al.*, 2019), while restoring central insulin signaling could prevent the onset of diabetes (Okamoto *et al.*, 2004). These findings provided the first evidence that selective central insulin resistance can cause obesity and other metabolic disturbances.

Humans with obesity show elevated plasma insulin levels, but reduced insulin concentrations in cerebrospinal fluid (Kern *et al.*, 2006; Heni *et al.*, 2014b). This suggests diminished insulin efficacy in the brain, potentially due to insulin resistance at the BBB (Verdile *et al.*, 2015). Moreover, in humans, central insulin resistance was first proposed in a neuroimaging studies using magnetoencephalography (MEG). Tschritter et al. demonstrated that adults with obesity exhibit absent brain responsiveness to exogenous insulin during hyperinsulinaemic-euglycaemic clamp, which is thought to result from disrupted insulin signaling in the hippocampus (Tschritter *et al.*, 2006).

Evidence points out that brain insulin signaling is already impaired in fetuses exposed to GDM during prenatal development. Animal studies indicate that hyperinsulinemia exposure can lead to brain insulin resistance in the fetus (Dearden *et al.*, 2020). During gestation, nutrients are delivered from the mother to the fetus via the placenta and umbilical cord, making the fetus highly susceptible to the maternal metabolic environment. In the case of GDM, elevated maternal glucose level results in an increase in fetal blood glucose through

placenta transfer, stimulating the fetal pancreas and resulting in fetal hyperinsulinemia (For review, see (Hufnagel *et al.*, 2022)). This hyperinsulinemia results in fetal macrosomia and raises the risk of these offspring developing obesity and T2D later in life (Hufnagel *et al.*, 2022). Weight gain following hyperinsulinemia exposure is often driven by increased food intake, as observed in children exposed to GDM (Luo *et al.*, 2021b). It is therefore plausible that chronic hyperinsulinemia exposure during fetal development affects brain maturation, as insulin signaling is necessary for proper brain development (Hufnagel *et al.*, 2022). Supporting this, fetal MEG studies showed a delayed brain response in fetuses with maternal GDM and higher obesity-related insulin resistance, after a glucose challenge (Linder *et al.*, 2014; Linder *et al.*, 2015). Furthermore, children exposed to GDM failed to show a normal brain reaction to glucose intake (Page *et al.*, 2019). These changes indicate that impaired brain insulin signaling resulting from intrauterine hyperinsulinemia exposure may also impact brain development in humans.

Figure 1 provides a comprehensive overview of the associations between obesity and insulin resistance.

1.2.2 Detection of Central Insulin Action in Human Brains

To detect central insulin action, various neuroimaging techniques can be used in combination with insulin administration techniques. Imaging modalities such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), electroencephalography (EEG), and MEG offer insights into how insulin influences brain metabolism and function (for review, see (Kullmann *et al.*, 2020a)). fMRI, in particular, measures blood-oxygen-level-dependent (BOLD) signals, enabling the detection of insulin-induced neural activity and functional connectivity during both resting state and different paradigms (Kullmann *et al.*, 2020a). For example, the visual food cue task is a widely used paradigm in which

participants view food images (vs. control images) to assess brain responses to food of varying caloric content.

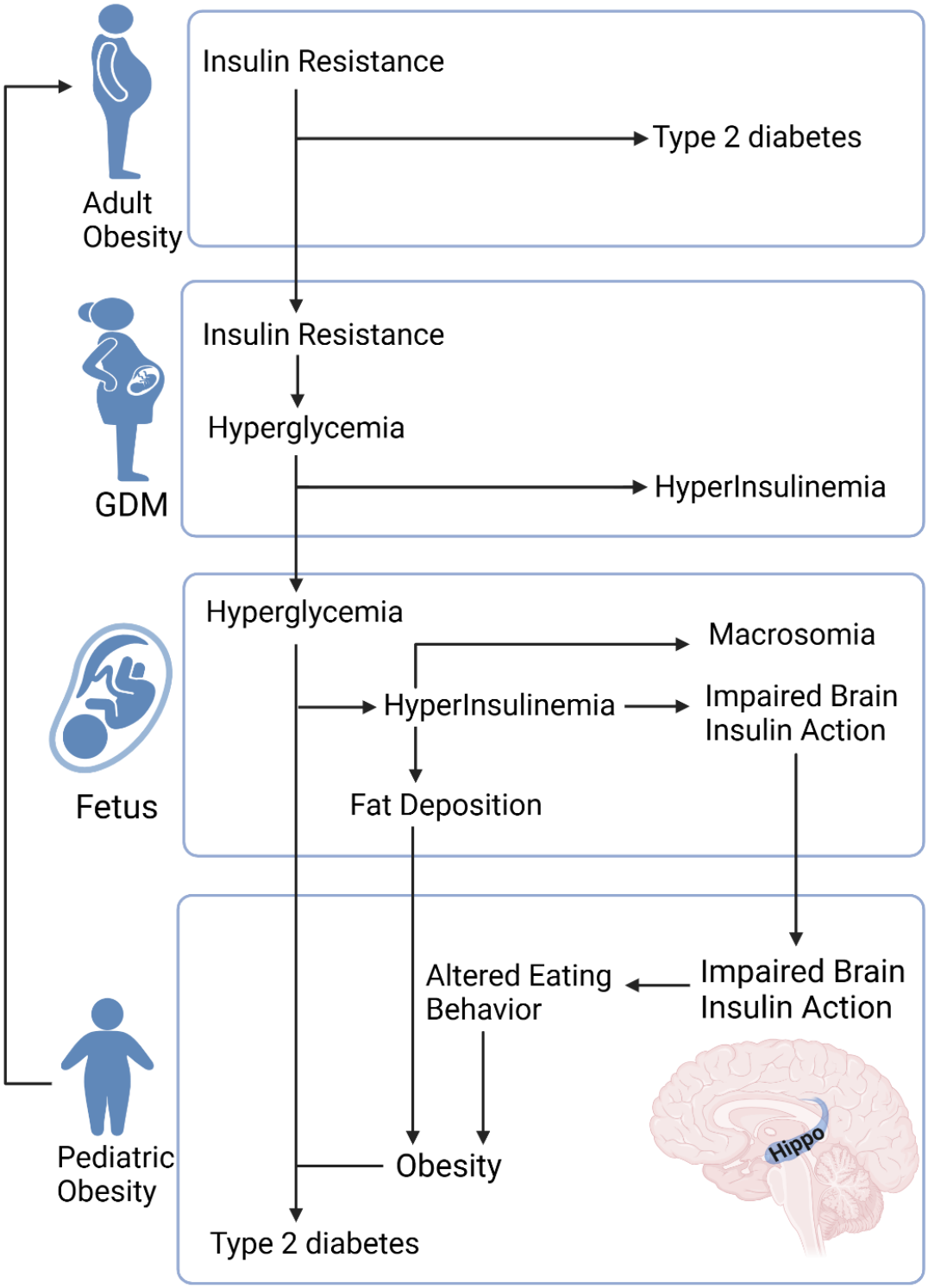

Figure 1 Overview of the associations between obesity and insulin resistance across lifespan. Figure was created with BioRender.com.
GDM, gestational diabetes mellitus; Hippo, hippocampus.

Insulin function in the human brain can be investigated using different techniques by means of endogenous insulin release, as the oral glucose tolerance test (oGTT) or exogenous insulin stimulation, using a hyperinsulinemic-euglycemic clamp or intranasal insulin, for example. Both the hyperinsulinemic-euglycemic clamp and oGTT influence insulin levels throughout the body, making it challenging to differentiate between centrally mediated and peripheral effects.
Intranasal insulin administration has been established as a reliable, non-invasive method for selectively investigating central insulin action. Only minimal amounts of intranasally administered insulin are absorbed systemically, minimizing the risk of peripheral side effects (Hallschmid, 2021). By utilizing the olfactory or trigeminal pathways, this technique allows insulin to bypass the BBB and reach the central nervous system (Dhuria *et al.*, 2010). In the olfactory pathway, insulin is thought to travel via extracellular routes through the intercellular spaces of the olfactory epithelium, ultimately reaching the olfactory bulb, from where it spreads to multiple brain regions, including the hippocampus (Edwin Thanarajah *et al.*, 2019a). Moreover, insulin also travels along the trigeminal nerves to reach the brainstem, as supported by findings from animal studies (Lochhead *et al.*, 2019). Thus, combining intranasal insulin administration with neuroimaging offers a powerful approach for studying insulin's central effects and may provide insights into therapeutic strategies for cognitive and metabolic dysfunctions.

1.2.3 Central Insulin and Brain Activity

Using intranasal insulin and fMRI, it is suggested that central insulin regulates peripheral energy balance primarily through its action on the hypothalamus, a main component of the homeostatic system. For instance, insulin-induced hypothalamic activity has been linked to improvements in peripheral glucose metabolism and insulin sensitivity in healthy adults (Heni *et al.*, 2014c; Heni *et al.*, 2017). Additionally, brain insulin has been shown to inhibit hypothalamic activity

(Kullmann *et al.*, 2013; Kullmann *et al.*, 2015), potentially resulting in decreased food consumption (Opstal *et al.*, 2017).

Another system that the central insulin interacts with is the mesocorticolimbic system. Insulin influences brain activity in regions such as the amygdala, striatum, and prefrontal cortex during resting state (Heni *et al.*, 2012; Kullmann *et al.*, 2013; Kullmann *et al.*, 2018). This activity is negatively related to food craving (Kullmann *et al.*, 2015). Furthermore, central insulin inhibited functional connectivity between the ventral tegmental area and nucleus accumbens during food valuation, which contributes to a reduction in food palatability rating (Tiedemann *et al.*, 2017). Notably, participants with insulin resistance show disruption in insulin action in the mesocorticolimbic system, companied with higher preference for palatable food (Heni *et al.*, 2012; Kullmann *et al.*, 2015; Tiedemann *et al.*, 2017; Edwin Thanarajah *et al.*, 2019b). These findings suggest that central insulin plays an important regulatory role in food reward behavior via mesocorticolimbic system (Kullmann *et al.*, 2020a).

1.3 The Hippocampus in Cognitive and Metabolic Regulation

1.3.1 Role of the Hippocampus in Memory and Learning

The hippocampus is an essential brain structure recognized for its fundamental role in various cognitive processes, particularly memory, learning and spatial navigation. It is widely recognized for its involvement in the formation, consolidation, and recall of various types of memories, including declarative memory (Ólafsdóttir *et al.*, 2018). Additionally, the hippocampus is crucial for spatial navigation, where it organizes relational memories and cognitive maps to guide movement through space (Redish and Touretzky, 1997; Eichenbaum, 2017). Beyond its well-established cognitive functions, the emerging evidence increasingly highlights its role in regulating food-related behaviors.

1.3.2 The Hippocampus in Appetite Regulation

Food intake regulation involves various brain regions within the mesocorticolimbic system, with the hippocampus integrating food-related episodic memories and sensations of hunger and satiety (for reviews, see (Kanoski and Grill, 2017; Buzsáki and Tingley, 2023)). Behavioral studies in healthy adults show that recalling recent meals can reduce future food consumption, while distractions during eating lead to increased intake later on (Higgs, 2002; Higgs *et al.*, 2008; Higgs and Woodward, 2009; Oldham-Cooper *et al.*, 2011). Amnesic adults struggle with interpreting signals of hunger and fullness, affecting their ability to regulate food intake (Hebben *et al.*, 1985; Rozin *et al.*, 1998). Moreover, the hippocampus is vulnerable to adverse conditions: even short-term exposure (4-7 days) to diets high in sugar and fat can impair learning and memory processes in both animals and humans (Winocur and Greenwood, 2005; Attuquayefio *et al.*, 2017; Stevenson *et al.*, 2020).

Neuroimaging studies have also identified the hippocampus as a key region involved in processing food cues and responding to sugar and postprandial hormones like insulin in both children and adults (Luo *et al.*, 2019; Kullmann *et al.*, 2020a; Jones *et al.*, 2021; Kanoski and Boutelle, 2022). Hippocampal activity during food cue processing differs between fasting and postprandial states, supporting its role in sensing interoceptive signals (Jones *et al.*, 2021). In individuals with obesity, both children and adults, increased hippocampal activity during food cue processing has been observed compared to lean counterparts, and these alterations predict food intake (Mestre *et al.*, 2017; Makaronidis and Batterham, 2018; Li *et al.*, 2021; Kösling *et al.*, 2022).

Moreover, the hippocampus is known to be particularly vulnerable to GDM exposure. Animals with chronic hyperinsulinemia in utero show impaired synaptic plasticity, and reduced neuronal density in the hippocampus in offspring, related to hippocampal insulin resistance (Golalipour *et al.*, 2012; Lotfi *et al.*, 2016; Vuong

et al., 2017; Schmitz *et al.*, 2018). Human studies validate these results, with children exposed to GDM and maternal obesity showing decreased hippocampal thickness and volume (Alves *et al.*, 2020; Lynch *et al.*, 2021).

Taken together, hippocampal dysfunction may impair the retrieval of meal-related memories or the processing of hunger and satiety signals, potentially contributing to overeating (for reviews, see (Kanoski and Grill, 2017; Parent *et al.*, 2022)).

1.3.3 Impact of Central Insulin on the Hippocampus

Although little is known about the hippocampal response to central insulin, some imaging studies involving intranasal insulin administration in adults have provided valuable insights. These studies have demonstrated alterations in hippocampal activation and functional connectivity in response to central insulin (Guthoff *et al.*, 2010; Zhang *et al.*, 2015; Kullmann *et al.*, 2017). Specifically, hippocampal activity was inhibited during a food cue task after intranasal insulin administration (Guthoff *et al.*, 2010). Furthermore, resting-state studies showed an increased functional connectivity between the hippocampus and prefrontal cortex following intranasal insulin administration compared to a placebo, observed in both metabolically healthy individuals and those with T2D (Zhang *et al.*, 2015; Kullmann *et al.*, 2017). This enhanced functional connectivity was also associated with reduced hunger feelings (Kullmann *et al.*, 2017), indicating an interaction between central insulin and the hippocampal network in appetite regulating.

1.4. Sex Differences in Central Insulin's Effect on Cognition and Metabolism

The effects of central insulin exhibit significant sex differences (for review, see (Hallschmid, 2021)). Behavioral research in both animal models and humans have shown that males and females respond differently to intranasal insulin, especially regarding food intake and memory. Males show reduced food consumption during fasting and weight loss following chronic insulin

administration (Clegg *et al.*, 2003; Hallschmid *et al.*, 2004; Benedict *et al.*, 2008), whiles females show enhanced hippocampus-dependent memory and decreased snack consumption after lunch following acute insulin administration (Benedict *et al.*, 2008; Hallschmid *et al.*, 2012).

To better understand the mechanisms behind these sex differences, animal studies have been conducted and suggested that low estrogen level is necessary for the anorexigenic effect of central insulin (Clegg *et al.*, 2006). However, human data do not fully support these findings in terms of food consumption and hippocampus-dependent memory (Krug *et al.*, 2010; Krug *et al.*, 2018), suggesting that estrogen might not be the sole factor impacting central insulin action in humans.

Despite considerable behavioral evidence for sex differences, neuroimaging studies have largely ignored the role of sex in central insulin action. Our previous works provided important insights, showing different central insulin action in the hippocampus and prefrontal cortex between males and females (Wagner *et al.*, 2022; Wagner *et al.*, 2023). We also demonstrated that variations in peripheral insulin sensitivity across the menstrual cycle may be regulated by central insulin, suggesting an interaction with sex hormones (Hummel *et al.*, 2023).

1.5. Aims of The Thesis

Given the dynamic nature of the developing brain, understanding the connection between pediatric obesity and brain alterations is critical to identify effective interventions. However, current findings are inconsistent, highlighting the need for systematic synthesis in this field. Moreover, it remains unclear how intrauterine hyperinsulinemia (exposure to GDM) and central insulin action impact the hippocampal network and its communication with other brain regions during the processing of high-caloric food cues. Similarly, the potential sex differences in these responses remain poorly explored.

Therefore, the aims of this thesis were to: 1) systematically review neuroimaging studies of brain alterations in children and adolescents with obesity (**Chapter 1**); 2) explore the associations between GDM exposure and hippocampal network alterations during food cue processing in children (**Chapter 2**); and 3) investigate the hippocampal network response to acute intranasal insulin, including potential sex differences and associations with eating behavior during food cue processing in adults (**Chapter 3**). To understand how hippocampal activation interacts with other brain regions in response to food images with varying caloric content, functional connectivity (hippocampal network) was measured during a food cue task.

2. Results and Discussion

2.1 Structural and Functional Brain Changes in Children and Adolescents with Obesity (Chapter 1)

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Minor Revision in the *Obesity Reviews* on 19 December 2024.

Chapter 1 systematically reviews neuroimaging studies examining brain alterations in children and adolescents with overweight and obesity. Structural changes, such as alterations in brain volume and cortical thickness, and functional changes, including neural activity and functional connectivity during resting state and food-/non-food paradigms, are discussed.

The mesocorticolimbic system emerges as particularly vulnerable to obesity in these populations. Obesity's impact on brain structure and function varies significantly across developmental stages. Mesolimbic regions show heightened activation in response to food-related rewards, while inhibitory control regions display decreased activation in children but inconsistent patterns in adolescents. Similar impacts are observed in children with parental metabolic conditions, such as maternal obesity and GDM, regardless of their current weight status. Additionally, non-pharmacological interventions, including exercise, eating behavioral strategies and weight-loss programs, demonstrate positive effects by increasing activation in inhibitory regions and reducing reward-related brain activity. The chapter concludes by identifying current gaps in pediatric obesity neuroimaging research and suggesting future research directions.

CHAPTER 1

Abstract

Obesity, particularly pediatric obesity has dramatically increased over the last three decades with a wide range of detrimental health outcomes, including negative consequences for brain neurodevelopment. The present article reviewed magnetic resonance imaging studies between January 2011 and March 2024 examining the brain's role in pediatric obesity, including parental influences and diverse interventions. A literature search identified 97 eligible MRI studies in the pediatric population. Findings suggest that altered brain structures and functions in pediatric obesity are strongly dependent on the developmental stage of children and adolescents. The function and structure of limbic regions, as the hippocampus, amygdala, and striatum, as well as the prefrontal cortex seem to be particularly affected by higher body mass index during development. In response to palatable foods, children and adolescents with excess weight have increased activation in reward-related regions and decreased activation in regions involved in interoceptive signal processing especially during decision processes. In addition, children of mothers with obesity and gestational diabetes mellitus show alterations in brain structure and function independent of their current obesity. Behavioral, exercise and weight-loss intervention studies showed promising effects on the brain with increased structural integrity, decreased brain responses to reward, and strengthened inhibitory brain responses in children and adolescence with excess weight after the intervention.

CHAPTER 1

1. Introduction

The global prevalence of overweight (Body Mass Index (BMI) percentile ≥ 85th) and obesity (BMI percentile ≥ 95th) among children and adolescents has risen dramatically, rising from 8% in 1990 to 20% in 2022 (WHO, 2024). Pediatric obesity could persist into adulthood, and it increases the risk of developing type 2 diabetes, cardiovascular disease, and cancer later in life (Kumar and Kelly, 2017).

During this developmental phase the brain undergoes rapid and dynamic development, characterized by heightened neuroplasticity (Tooley *et al.*, 2021). Magnetic resonance imaging (MRI) enables the estimation of brain size, microstructure, and the function of specific systems throughout childhood and adolescence. It has been shown that the volume of cortical grey matter generally develops in an “inverted U” pattern, which is increasing during early childhood and declining in post adolescence, with latest maturation in the prefrontal cortex (for reviews, see (Lenroot and Giedd, 2006; Konrad *et al.*, 2013; Herting and Sowell, 2017)). Mesolimbic regions, such as basal ganglia, and hippocampus/ amygdala exhibit the same developmental pattern with peaking time at 10 and 14 years respectively (Lenroot and Giedd, 2006; Tamnes *et al.*, 2018; Russell *et al.*, 2021). Cortical thickness keeps decreasing, and the integrity of white matter keeps increasing throughout the whole childhood and adolescence (Herting and Sowell, 2017). Generally, both structural and functional studies indicate an earlier maturation in the mesolimbic reward regions, while the prefrontal control system develops later (Konrad *et al.*, 2013; Herting and Sowell, 2017).

Several neurobehavioral hypotheses for the development of obesity have been proposed, even though they are not exclusively focused on pediatric obesity (for review see (Stice and Yokum, 2021)). For example, the incentive sensitization hypothesis suggests that overconsumption of high-calorie food leads to increased response to food rewards in reward (e.g., striatum, amygdala,

hippocampus, orbitofrontal cortex, insula) and attentional regions (e.g., occipital cortex, fusiform gyrus, frontal operculum, anterior cingulate cortex) via food reward learning, subsequently promoting overeating. The reward surfeit hypothesis posits that a hyperactivity to high-calorie food tastes in the reward system contributes to overeating and obesity. In addition, the inhibitory control deficit hypothesis proposes that lower activation in inhibitory control regions (e.g., ventral lateral prefrontal cortex, dorsolateral prefrontal cortex) in response to immediate food rewards results in overeating. Despite relying on different circuitries, all these hypotheses of obesity imply an imbalance of reward and cognitive control systems.

The aim of this review is to provide a detailed insight into neuroimaging research (i.e., using structural and functional magnetic resonance imaging (MRI and fMRI)) examining the neural alterations related to pediatric obesity. Moreover, we consider how maternal or paternal metabolic status influences obesity onset in the offspring. Lastly, we review available non-pharmacological intervention strategies and their effect on brain structure and function in pediatric obesity. We will also evaluate the evidence for the different hypotheses regarding obesity.

2. Materials and Methods

2.1 Search strategy

We reviewed MRI research on excess weight related neuro-alterations among children and adolescents. To identify relevant studies, we searched the online database PubMed and Web of science to include articles published between January 2011 and March 2024. Search terms are given in the Supplementary Text. We screened the identified studies to see if they matched the review topic by reading the title and abstract and examined the remaining articles in detail. The study selection process is illustrated in the **Supplementary Figure 1**.

2.2. Selection criteria and data extraction

In the current review, inclusion criteria for studies were as follows: 1) empirical research, 2) child or adolescent samples (1-18 years old), 3) MRI methodology (e.g., fMRI) was used, 4) study sample includes children or adolescents with excess weight (BMI ≥ 25 kg/m2; BMI percentile ≥ 85th). The following studies were excluded: 1) review articles, case studies or meeting abstracts, 2) participants with type 2 diabetes, Prader–Willi syndrome, and psychiatric/ neurological disorders, 3) studies not written in English.

Extracted data included information regarding sample characteristics (i.e., age, sample size, Tanner stage, weight status, gender, exclusion criteria), MRI methods (including MRI modality, task description, covariates), interventions used, and a summary of key findings from the extracted data.

2.3. Selected publications

Thus far, 97 publications in total met the inclusion criteria. Most studies (n = 63) investigated effect of obesity on brain structure and neural activity in children and adolescents (n = 23 assessed structural differences (in both grey and white matter), n = 5 assessed microstructural differences in white matter, n = 1 detected both structural and white matter microstructural differences, n = 1 detected structural, white matter microstructural differences and intrinsic neural network using rs-fMRI, n = 1 detected structural differences and intrinsic neural network, n = 25 examined neural activity during different paradigms, n = 1 examined both structure and neural activity, n = 6 examined intrinsic neural networks). In **Table 1** and **2**, we summarize the characteristics of these studies. **Figures 1** and **2** illustrate the major structural and functional brain alterations in children and adolescents with excess weight. 9 studies investigated the role of parental metabolic status on pediatric obesity. 25 studies examined effects of physical fitness on brain health and different non-pharmacological interventions on the brains. The characteristics of these studies are summarized in the **Supplementary Table 1** and **2**, respectively. **Supplementary Figure 2**

describes the brain alterations in children and adolescents with excess weight after diverse interventions. We use the phrase excess weight for all participants above the normal weight range.

3. Results

3.1. Evidences of structural differences in the brain of children with excess weight

Obesity impacts the brain structure in children. Previous studies measured different metrics, including volume, cortical thickness and T2 relaxation time, to assess brain structural alterations in children with excess weight. Volumetric alterations in brain areas indicate changes in either the number or the size of cells in these areas (de Sousa and Proulx, 2014). Cortical thickness measures gray matter width, which is determined by synaptic density, as well as intracranial myelination (Tahedl, 2020). In addition, T2-weighted signal intensity can provide insights into changes of brain tissue properties, by detecting variations in water content, where hyperintense signals reflect gliosis (Bitar *et al.*, 2006; Sewaybricker *et al.*, 2019).

Our review includes 26 studies addressing structural alterations associated with pediatric obesity. Volumetric alterations in mesolimbic regions, such as hippocampus, amygdala, and basal ganglia (divided into globus pallidum and striatal divisions) have been linked to obesity, but results are inconsistent. For example, compared to their lean peers, children with excess weight showed lower volumes in the hippocampus and amygdala (Bauer *et al.*, 2015; Mestre *et al.*, 2017; Jiang *et al.*, 2023), while adolescents with excess weight (13 - 14 years) exhibited higher volumes in these regions (Moreno-López *et al.*, 2012; Perlaki *et al.*, 2018). Similarly, a positive correlation between BMI and the volume of the globus pallidum and nucleus accumbens (NAcc, i.e. ventral striatum) was found in adolescents (de Groot *et al.*, 2017; Perlaki *et al.*, 2018). However, other studies found no correlation between the volume of the amygdala, hippocampus and

obesity measures among older adolescents (14 - 16 years) (de Groot *et al.*, 2017; Mestre *et al.*, 2020). The observed inconsistency in mesolimbic regions between children and adolescents might arise from obesity disrupting the typical developmental pattern, characterized by an initial increase in the volume of these regions during early childhood, followed by a decline during adolescence (Herting and Sowell, 2017). However, longitudinal studies in both children and adolescents (spanning puberty) with excess weight showed a greater reduction in the volume of the amygdala, hippocampus and caudate (dorsal striatum) after a 2-year (Jiang *et al.*, 2023) and 3-year (Hashimoto *et al.*, 2015) follow-up respectively, suggesting a potential detrimental impact of obesity on brain growth. Notably, studying a wide age range from childhood to adolescence, could mask interaction effects that change throughout the developmental process. Interestingly, better sleep and longer breastfeeding duration were associated with larger hippocampus in children with excess weight (Migueles *et al.*, 2021; Higgins *et al.*, 2022).

The structure of the prefrontal cortex (PFC), a critical brain region related to executive function, has been widely studied in children and adolescents, employing large sample sizes. For instance, children with higher BMI or waist circumference exhibited decreased volume and cortical thickness in several regions of the PFC (Laurent *et al.*, 2020; Ronan *et al.*, 2020; Brooks *et al.*, 2023; Cui *et al.*, 2023; Hall *et al.*, 2023; Kaltenhauser *et al.*, 2023; Zhang *et al.*, 2023). Lean mass index positively predicted PFC volume (Gracia-Marco *et al.*, 2020). Furthermore, the reduced volume and thickness of the PFC partially mediated the inverse relationship between BMI and executive function (e.g., working memory) (Laurent *et al.*, 2020; Ronan *et al.*, 2020; Sakib *et al.*, 2023). Additionally, children with excess weight showed a greater reduction of PFC volume and thickness over 2-years of development (Jiang *et al.*, 2023; Kaltenhauser *et al.*, 2023). Conversely, older adolescents exhibited a thicker PFC with increased

visceral abdominal fat (Saute *et al.*, 2018), implying a potential reversal in the developmental pattern of cortical thickness due to obesity. According to the robust correlations supported by large sample sizes and replicated results, the observed link between obesity and the size and morphology of the PFC, this region is likely to be vulnerable to the effect of pediatric obesity. The delayed maturity of the PFC compared to other brain regions during childhood and adolescence could be the reason (Laurent *et al.*, 2020). However, given that the majority of studies were cross-sectional in nature, a clear causal relationship between brain development and obesity remains elusive. In addition, inflammatory biomarkers (Adelantado-Renau *et al.*, 2019) and adverse early life factors (Solis-Urra *et al.*, 2019) were also related to decreased PFC volume in children with excess weight.

T2 relaxation time was used to investigate tissue properties, especially gliosis resulting from obesity, in the hypothalamus (homeostatic system) and hippocampus among adolescents. For example, adolescents with higher BMI exhibited longer T2 relaxation time in the hypothalamus (Sewaybricker *et al.*, 2019; Sewaybricker *et al.*, 2021b), suggesting the presence of hypothalamic gliosis, a response of the central nervous system to injury caused by obesity or high fat diet (Sewaybricker *et al.*, 2019). In addition, hypothalamic gliosis predicted weight gain over one year in adolescents with overweight but not with obesity, suggesting that gliosis could potentially precede the development of obesity (Sewaybricker *et al.*, 2021a). On the contrary, a negative association between BMI z-score and T2-relaxation time was found in the hippocampus (Mestre *et al.*, 2020). Unlike gliosis, a decreased T2 relaxation time in the hippocampus might be due to the accumulation of macromolecules and lipids resulting from a high fat diet, resulting in heightened tissue viscosity (Autti *et al.*, 2007). However, there remains a research gap regarding the tissue properties in children with excess weight. Moreover, exploring additional brain regions, such

as other limbic regions, could provide deeper insights into brain alterations related to pediatric obesity.

3.1.1. Summary

According to these studies, children and adolescents with excess weight have altered volume, cortical thickness, or tissue properties mainly in the limbic regions, PFC and hypothalamus. Although the results are mixed, multiple studies found decreased cortical thickness and volume in the PFC, accompanied with diminished executive function. Volumetric alterations in the hippocampus, amygdala and basal ganglia are strongly dependent on developmental stage, with decreases observed in children and increases in adolescents (**Figure 3a**).

3.2. Evidences of white matter microstructural differences in the brain of children with excess weight

Besides alterations in brain size, obesity-related changes in white matter microstructure have also been investigated using diffusion tensor imaging (DTI), a neuroimaging technique that detects water diffusion at the cellular level (Alexander *et al.*, 2007). Fractional anisotropy (FA) is the primary indicator of white matter integrity (Alexander *et al.*, 2007).

We identified 7 studies linking excess weight to altered white matter integrity in children and adolescents. Association fibers, which connect the cortical regions within same hemisphere, seem to be susceptible to obesity. For example, children with obesity aged 7-11 years showed higher FA in the association fibers, as well as lower FA in the superior cerebellar peduncle, compared to their lean peers (Ou *et al.*, 2015; Augustijn *et al.*, 2018). A structural network analysis revealed stronger connections within the ventral and dorsal striatum, as well as greater involvement of the precentral gyrus in this age group (Augustijn *et al.*, 2019b), suggesting that obesity impacts both the reward network and motor cortex. However, this study did not examine behavioral data on reward processing or motor skills. Larger head circumference at birth and worse

household socioeconomic status also influence the maturation of association fibers in these children (Solis-Urra *et al.*, 2022; Li *et al.*, 2023b). In older children and adolescents, however, a negative association was observed between BMI and FA in the association fibers and callosal fibers, with BMI negatively predicting FA in these fibers over a 2-year development (Alarcón *et al.*, 2016; Kaltenhauser *et al.*, 2023). Obesity may contribute to the inconsistency in FA values observed in the association fibers during development by disrupting the typical linear increase observed from childhood to adulthood. Additionally, FA values are determined by water diffusivity along three distinct axes, each of which can independently impact FA. Consequently, alterations in diffusion along these directions could also result in varied findings. However, given the limited research, especially in early adolescence, more studies are necessary to replicate the current findings.

3.3. Evidences of functional differences in the brain of children with excess weight

By assessing changes in the paramagnetic properties of hemoglobin, which depend on blood-oxygen levels, fMRI, including task-based fMRI and rs-fMRI, can infer local neuronal activity and functional connectivity (Carnell *et al.*, 2012).

3.3.1. Resting state fMRI

In comparison to task-based fMRI, resting state functional connectivity (rs-FC) provides insight into intrinsic neural networks (Borowitz *et al.*, 2020).

There are a total of 8 studies investigating rs-FC in children and adolescents with excess weight. They showed altered rs-FC between regions related to cognition and reward processing, with alterations in rs-FC between reward-related regions. These alterations are notably influenced by the developmental stage. For example, adolescents with excess weight showed decreased rs-FC between cognitive regions (e.g., hippocampus, dorsolateral PFC) and reward regions (e.g., caudate, orbitofrontal cortex) (Moreno-Lopez *et al.*, 2016; Martín-

Pérez *et al.*, 2019; Borowitz *et al.*, 2020); also increased FC among reward regions (e.g., pallidum, orbitofrontal cortex) (Martín-Pérez *et al.*, 2019; Borowitz *et al.*, 2020). While children with excess weight exhibited the opposite connectivity patterns (Black *et al.*, 2014; Pujol *et al.*, 2021). Reward regions are relatively more developed in adolescence than cognitive regions (Casey and Jones, 2010), and these reward areas might simply be underdeveloped in younger children, resulting in a lack of strong connections (Casey *et al.*, 2000; Borowitz *et al.*, 2020). However, these studies investigated connectivity patterns between specific regions based on researchers' hypotheses, rather than exploring effects of obesity on brain wide connectivity. Only two studies using network analysis, showed a negative association between BMI and rs-FC within salience, executive control and default mode networks in children with excess weight (Brooks *et al.*, 2023; Kaltenhauser *et al.*, 2023). Whether these brain alterations predict food craving behavior or subsequent weight gain was not examined. Early life factors, such as birth weight and breastfeeding were also associated with hippocampal FC in children with excess weight (Solis-Urra *et al.*, 2023).

In sum, the available literature suggests that adolescents with excess weight have weaker connectivity between regions involved in cognition and reward, and stronger connectivity between reward regions. Children with excess weight show an opposite pattern.

3.3.2. Task-based fMRI

A variety of different tasks were used in relation to pediatric obesity. Specifically, food-related paradigms including a visual food cue task (n = 5) (Yokum and Stice, 2013; Jastreboff *et al.*, 2014; Adam *et al.*, 2015; Masterson *et al.*, 2019; Roth *et al.*, 2019), food specific go/no-go task (n = 1) (Jensen *et al.*, 2019), tasting (n = 4) (Boutelle *et al.*, 2015; Bohon, 2017; Mestre *et al.*, 2017; Yokum and Stice, 2023), food specific attention network task (n = 1) (Yokum *et*

al., 2011), food choice task (n = 2) (Moreno-Padilla *et al.*, 2018; van Meer *et al.*, 2019), food and physical activity decision task (n = 1) (Lim *et al.*, 2023) and a food commercial trigger (n = 5) (Bruce *et al.*, 2013; Burger and Stice, 2014; Yokum *et al.*, 2014; Rapuano *et al.*, 2016; Gearhardt *et al.*, 2020) were used to examine eating behavior related brain response in children and adolescents with excess weight. Furthermore, non-food paradigms including a monetary incentive delay task (n = 1) (Navas *et al.*, 2018), modified card-guessing task (n = 2) (Adise *et al.*, 2018; Adise *et al.*, 2019), risky-gains task (n = 2) (Delgado-Rico *et al.*, 2013; Mata *et al.*, 2015), social decision-making task (n = 1) (Verdejo-García *et al.*, 2015), and a chatroom task (n = 1) (Jensen *et al.*, 2022) were used to investigate neural activity.

3.3.2.1. Food related paradigms

The chronic overconsumption of calories over the life course plays a major role in obesity (Nielsen *et al.*, 2002). Hence, brain responses to high-calorie food cues are investigated to evaluate neural mechanisms of eating behavior. Children and adolescents with excess weight showed higher response to visual high-calorie food stimuli (vs. non-food) in reward-processing brain regions such as the medial orbitofrontal cortex (OFC), ventral striatum, dorsal striatum, amygdala, substantia nigra/ventral tegmental area and insula than their lean counterparts (Jastreboff *et al.*, 2014; Roth *et al.*, 2019), aligning with the incentive sensitization hypothesis of obesity. The responses in the reward regions were also positively correlated with leptin level and negatively correlated with insulin sensitivity (Jastreboff *et al.*, 2014; Adam *et al.*, 2015), supporting the role of these hormones in regulating eating behavior. However, behavioral data such as food craving or food intake were not assessed in these studies. Additionally, different cognitive strategies, such as considering benefits of not eating when confronted with high-calorie food cues, resulted in increased activation in the inhibitory control regions and decreased activation in the attention-related regions among adolescents,

independent of their BMI (Yokum and Stice, 2013). Despite its small sample size, the study could provide a theoretical framework for developing effective interventions, such as cognitive training. Interestingly, sleep restriction increased food cue-related reward processing independent of weight status during a food specific go/no-go task (Jensen *et al.*, 2019).

In addition, elevated responses in regions related to reward, gustation and attention to appetitive taste (vs. water) predicted increased eating in absence of hunger in children with excess weight (Boutelle *et al.*, 2015; Bohon, 2017; Mestre *et al.*, 2017; Yokum and Stice, 2023), suggesting an increased sensitivity of gustation. These results support the reward surfeit hypothesis of obesity, highlighting the significance of brain responses to food taste in the development of obesity.

An attention bias to food in adolescents with excess weight was found as well (Yokum *et al.*, 2011). Specifically, during a food-specific attention task, adolescents with higher BMI not only exhibited a faster response to food stimuli, but also a hyperactivity in the reward and attentional regions to appetitive food pictures. Moreover, these elevated responses predicted weight gain 1-year later (Yokum *et al.*, 2011). These findings also support the incentive hypothesis of obesity, emphasizing the role of reward and attentional regions for weight gain. Notably, only female adolescents were included in this study.

Caloric intake is also controlled by decisions (Delgado-Rico *et al.*, 2013). Adolescents with higher BMI exhibited decreased activation in the inhibitory control regions when choosing appetizing food and physical activity images (van Meer *et al.*, 2019; Lim *et al.*, 2023), which is consistent with the hypothesis of an inhibitory control deficit. Higher response in the attentional regions during food choice predicted greater weight gain per year (van Meer *et al.*, 2019), aligning more closely with the incentive sensitization hypothesis. However, increased response in the inhibitory control regions during food choice was found in older

adolescents (Moreno-Padilla *et al.*, 2018). Again, the inconsistent results could arise from the interaction between obesity and developmental trajectory. For example, the older the adolescent, the more mature the inhibitory control regions, potentially prompt more deliberate attempts to suppress appetite resulting a higher reaction to food rewards (Moreno-Padilla *et al.*, 2018). Alternatively, studies with smaller sample sizes could contribute to the replication crisis, serving as another potential reason.

Brain responses to food commercials (e.g., food advertisements or logos), identified as significant contributors to children's eating behavior leading to obesity (Story and French, 2004), were also examined, even though they are not standardized paradigms. Greater response to food commercials in reward and attentional regions predicted greater subsequent high-calorie food intake and weight gain one-year later in children and adolescents (Burger and Stice, 2014; Yokum *et al.*, 2014; Rapuano *et al.*, 2016; Gearhardt *et al.*, 2020). The effects of excess weight, however, were not specifically investigated in these studies. Other studies showed that children with excess weight had increased activation in attentional regions and decreased activation in inhibitory control regions compared to their lean peers (Bruce *et al.*, 2013; Masterson *et al.*, 2019). These results are consistent with both incentive sensitization and inhibitory control deficit hypotheses of obesity. It appears that the rewarding properties of food commercials affect all children, but the impact is more pronounced in those with excess weight, potentially diminishing their self-control. Reducing exposure to food commercials in their daily life could be an effective intervention.

3.3.2.1.1. Summary

Taken together, children and adolescents with excess weight show hyperactivity in regions related to reward, gustation and attention to external food cues and taste. These responses are predictive for subsequent overeating and weight gain. We therefore suggest that current findings are more consistent with

the incentive sensitization hypothesis of obesity, emphasizing the role of reward regions in the development of pediatric obesity. The findings regarding activation in inhibitory control regions were mixed (Figure 3b).

3.3.2.2. Non-food paradigms

To examine whether the association between pediatric obesity and hyperactivity in reward-related regions is specific to food or general to all rewards, studies employed paradigms involving non-food (e.g., monetary) rewards. Adolescents with higher body fat had decreased activation in the somatosensory cortex to monetary reward feedback (Navas *et al.*, 2018). However, no association was observed between weight status and the brain's response to either monetary or food rewards in children (Adise *et al.*, 2018; Adise *et al.*, 2019). Given that children's brains exhibit less maturity compared to those of adolescents, it is possible that the effects of weight status on neural processing to food vs. monetary reward was not fully distinguished in children (Adise *et al.*, 2019). Different task designs in children versus adolescents could be another possible reason for the inconsistent results.

Maximizing reward at the cost of risk seems to characterize the adolescent brain (Ernst and Fudge, 2009). Among adolescents with excess weight, decreased activation in the risk-signaling region (i.e., insula) to risky choices predicted reduced interoceptive sensitivity and increased external eating. Increased activation in the reward region than their lean peers was observed as well (Delgado-Rico *et al.*, 2013; Mata *et al.*, 2015). These findings may suggest a higher focus on the reward properties than on the possible long-term risks associated with a decision in adolescents with excess weight, resulting in a propensity to choose high-calorie foods in their daily life.

In addition, neural responses in adolescents with excess weight have been found to be influenced by social factors, stress and sleep. Obesity in adolescents decreased brain responses to unfair monetary offers in reward and emotional

regions during a social decision-making task, suggesting reduced emotional monitoring of social unfairness (Verdejo-García *et al.*, 2015). Social stress was also related to obesity. For instance, among girls with excess weight who were sleep deprived, both positive and negative peer evaluations were linked to increased brain activity in emotion-related regions. This was interpreted as them paying more attention to social feedback (Jensen *et al.*, 2022).

In sum, no relationship was observed between obesity and brain responses to other form of reward in children, but a negative correlation was identified in adolescents. Decreased activation in interoceptive signal processing regions, and increased activation in reward regions during decision-making were also found in adolescents with excess weight. However, caution is warranted in interpreting these results due to the limited number of studies and the variability in task designs.

3.4. The role of parents on brain's function and structure of children

Evidence indicate that genetics, epigenetics, shared environment and other factors contribute to weight gain (Faith *et al.*, 1999). Parental obesity is a reliable predictor of offspring obesity risk in childhood, adolescence, and adulthood (Rath *et al.*, 2016). Exposure to maternal gestational diabetes mellitus (GDM) and obesity in utero also increase risk for obesity in offspring (Page *et al.*, 2014). Our review provided 9 studies addressing the role of mothers on the brain response of children and adolescents at high risk for excess weight.

3.4.1. Parental obesity and gestational diabetes mellitus

Even lean adolescents with obese mother showed a reduced volume or cortical thickness in the somatosensory and taste cortex, as well as inhibitory control regions, compared to lean peers of normal weight mothers (Thapaliya *et al.*, 2021). Independent of their current adiposity, children also showed weaker activation in inhibitory control regions in response to food cues, suggesting weakness in inhibitory control circuitry could play a role in the intergenerational

effects of obesity (Carnell *et al.*, 2017; Luo *et al.*, 2021a). Only one study thus far has investigated paternal influence on children's brain, and, no relationship was found between children food cue reactivity and paternal BMI (Luo *et al.*, 2021a). Risk for developing obesity also derives from exposure to maternal metabolic disorders in utero. For example, the volume or radial thickness of the hippocampus, middle frontal gyrus and superior temporal gyrus in children exposed to maternal pre-pregnancy obesity and GDM was decreased independent of their current BMI (Alves *et al.*, 2020; Lynch *et al.*, 2021; Luo *et al.*, 2023). Similarly, independent of children's adiposity, GDM exposure increased hypothalamic cerebral blood flow and gliosis, as well as OFC activation in offspring (Page *et al.*, 2019; Luo *et al.*, 2021b; Chandrasekaran *et al.*, 2022), suggesting that both, homeostatic and mesolimbic areas might be affected by GDM exposure. However, these studies are based on specific regions of interest. Future studies based on other hypotheses, such as dysfunction of inhibitory control system in children exposed to GDM, are necessary.

Collectively, the included studies suggest that independent of their current adiposity, children of mothers with excess weight have a hypoactive inhibitory control circuitry, while those who are exposed to early GDM show hyperactivity in the homeostatic and reward system. Moreover, maternal metabolic disorders may induce hippocampal structural alteration in children. However, we are unable to fully depict the impact of parental metabolic status on children's brains, as studies examining children with normal weight but obese parents were not included in this review.

3.5. Non-pharmacological interventions against pediatric obesity

Several interventions have been developed to prevent and treat pediatric obesity. These interventions target physical activity, eating behavioral adaptations, or a combination of these. Our research provided 12 studies investigating the relationship between physical fitness and brain

function/structure in children and adolescents with excess weight, and other 13 longitudinal studies addressing the effects of different interventions on their brain, including exercise.

3.5.1. Physical fitness and exercise intervention

Physical fitness, investigated by physiological parameters (i.e., cardiorespiratory/ speed-agility/ muscular fitness) and questionnaires, was positively related to brain volume, cortical thickness, global FA, node clustering and connectivity of the resting-state networks in children and adolescents with excess weight (Esteban-Cornejo *et al.*, 2017; Esteban-Cornejo *et al.*, 2019a; Esteban-Cornejo *et al.*, 2019b; Rodriguez-Ayllon *et al.*, 2020a; Rodriguez-Ayllon *et al.*, 2020b; Alves *et al.*, 2021; Brooks *et al.*, 2021; Esteban-Cornejo *et al.*, 2021; Logan *et al.*, 2022; Adelantado-Renau *et al.*, 2023; Cadenas-Sanchez *et al.*, 2023; Haapala *et al.*, 2024). The above findings indicate that brain development may benefit from physical activity. Hence, exercise interventions are used to improve the brain health in children and adolescents with excess weight.

In children with excess weight, even though cognitive ability was improved, no effect of a 20-weeks aerobic and resistance exercise intervention on brain volume was found, which may result from insufficient intervention time or alterations restricted to the cellular or molecular level (Ortega *et al.*, 2022). Interestingly, effects of exercise on the white matter microstructure were found. For example, compared to sedentary group, an 8-months aerobic exercise intervention increased FA in the fronto-temporal and fronto-parietal fiber tracts of children from baseline, a change that was also related to higher attendance in the exercise (Krafft *et al.*, 2014b; Schaeffer *et al.*, 2014). In the same exercise program, reduced synchrony in default mode network, executive control network and motor network; altered brain activation in cognitive processing regions during an antisaccade/flanker task; and increased cognitive performance were also found after intervention (Krafft *et al.*, 2014a; Krafft *et al.*, 2014c). This may

suggest an improvement in brain specialization and efficiency with exercise (Krafft *et al.*, 2014a; Krafft *et al.*, 2014c). Similar results were shown in another 3-month aerobic exercise intervention study (Davis *et al.*, 2011). Whether these children lost weight was not reported in above studies.

3.5.2. Eating behavioral strategies

The adaptation of eating behavior has been shown to be an effective strategy in managing weight (Carnell *et al.*, 2013). For example, compared to breakfast-skipping day, adolescents' brain responses to food (vs. non-food) cues in reward/motivation regions was decreased following a 6-days breakfast consumption. The brain activation in these regions was also positively related to appetite (Leidy *et al.*, 2011). Another study showed a reduced food cue reactivity in reward and visual attention regions following glucose consumption after a 6-months food intake reduction device training, which reduces portion size and eating speed by feedback technique (Hinton *et al.*, 2018). Even though findings from these studies may suggest a reduction of subsequent food intake, this parameter was not investigated in these studies, nor was weight loss. Notably, the reduced sensitivity to food rewards observed post-intervention supports the incentive sensitization hypothesis of obesity, which highlights hypersensitivity in reward regions promoting overeating and obesity. A decrease in sensitivity to food rewards could suggest a normalization of the reward system, similar to that observed in children with normal weight.

3.5.3. Weight-loss program intervention

Weight loss interventions, combining exercise with dietary restriction, cognitive behavioral therapy, family management etc., have been used commonly to manage weight and reverse the effects of obesity on the brain. For example, after a 5-months combined intervention including exercise, dietary restriction and cognitive behavioral therapy, there was not only a significant weight loss, but cerebellar cortex and total grey matter volume were increased

among children (Augustijn *et al.*, 2019a). In addition, adolescents who exhibited a greater increase in insula activation during a risky-gain task from baseline to post a 12-week similar intervention lost more weight. This implies that during risky decision-making, the insula displayed heightened responsiveness in individuals who achieved greater weight loss, potentially suggesting a normalization of the interoceptive system (Mata *et al.*, 2016). Similarly, adolescents with greater reductions in BMI showed a normalization of the reward system after exercise and dietary intervention as well (Kinder *et al.*, 2014). Moreover, decreased activation after a meal and lower rs-FC in the appetitive regions before intervention predicted greater weight loss after 6 months (Schur *et al.*, 2020) and 3months (Martín-Pérez *et al.*, 2020), respectively. This may suggest a predictive role of the reward system for successful weight loss.

In summary, exercise is the most commonly used intervention form to improve brain health in children and adolescents with excess weight with widespread effects on brain structure and function. Eating behavioral adaptations result in decreased brain reward responses. A low response to reward and high response to risky decision making seem to predict the success of weight loss.

4. Discussion

Pediatric obesity is a concerning public health burden that needs to be addressed. MRI studies in children and adolescents with excess weight provide evidence of obesity-related alterations in brain function and structure. Findings suggest that altered brain structure and function in pediatric obesity are strongly dependent on the age or developmental stage of the children and adolescents studied (**Figure 3**). Specifically, limbic regions, as the hippocampus, amygdala, striatum exhibit decreased volume in children with excess weight, contrasting with increased volume in adolescents. Furthermore, studies consistently report decreased volume and cortical thickness in the PFC. In response to palatable foods, children and adolescents with excess weight have increased activation in

reward and attention-related regions and decreased activation in regions involved in interoceptive signal processing especially during decision making processes. Activation patterns in inhibitory control regions vary inconsistently across studies. We suggest that these findings support the incentive sensitization hypothesis, given the hyperactivity of primarily reward related regions and predictive role of these regions in future weight gain. In addition, children of mothers with obesity and GDM show similar alterations in brain structure and function independent of their current adiposity. Furthermore, exercise, eating behavioral adaption and weight-loss intervention studies showed promising effects with increased structural integrity, decreased brain reward responses in children and adolescence with obesity after the intervention. However, post-intervention weight loss in studies of exercise and eating behavioral adaption was not reported. In weight loss programs, a decreased brain response to reward and an interoceptive system sensitive to risky decision making predicts the success of weight loss. The findings summarized in this review can provide a theoretical framework for developing more effective interventions.

Obesity has a strong genetic basis, encompassing genes linked to early-onset monogenic obesity in pediatric populations and polygenic obesity, many of which are expressed in the brain (Saeed *et al.*, 2024). These obesity-related genes are thought to influence body weight primarily through centrally-mediated effects (Saeed *et al.*, 2024). A recent review highlighted two distinct neural pathways in the brain's regulation of energy balance in monogenetic and polygenic forms of obesity, based on gene expression in specific brain regions (Saeed *et al.*, 2024). For monogenic obesity, such as leptin deficiency and Prader-Willi syndrome, an impaired hypothalamic pathway involved in appetite control has been identified (Saeed *et al.*, 2024). However, neuroimaging studies in children suggest that structural and functional alterations extend beyond the hypothalamus, impacting other areas, including the mesolimbic circuitry (Wu *et*

al., 2022; Huang *et al.*, 2024a; Huang *et al.*, 2024b). In contrast, polygenic obesity is thought to involve disruption in reward processing pathway (Saeed *et al.*, 2024), with many obesity-associated genes being highly expressed in the mesolimbic circuitry (Ndiaye *et al.*, 2020). This aligns with the incentive sensitization hypothesis and is supported by MRI studies in pediatric populations, which reveal reduced brain volume in regions within this circuitry (Kennedy *et al.*, 2019; Morys *et al.*, 2023).

The review indicates neural changes in children and adolescents with excess weight; nevertheless, several limitations persist. There is a considerable variation in sample sizes, such as from 12 (Adam *et al.*, 2015) to 230 (Pujol *et al.*, 2021) among fMRI studies. It is crucial to question whether findings from these smaller studies accurately represent the effects of population-level alterations. Power analyses should be conducted to ensure appropriate sample sizes for research. In addition, variations in fasting durations before task-based fMRI measurement (from satiety to 6 hours), and exclusive focus on specific sex (Yokum *et al.*, 2011; Adam *et al.*, 2015; Lim *et al.*, 2023) require caution while interpreting certain findings. Moreover, reward-related tasks were typically performed in children, whereas adolescents were engaged in tasks related to attention and decision-making. This could also lead to skewed findings. Furthermore, limited longitudinal studies prevents us from reaching a conclusion regarding long-term brain outcomes resulting from disruption by obesity. Finally, despite evidence showing the relationship between pubertal measures/hormones and brain structuring during adolescence (Blakemore, 2012), no studies have investigated the specific impacts of pubertal status on the brains of children and adolescents with excess weight.

Future research may benefit from task designs that focus more specifically on inhibitory control, to examine how self-regulation is affected by appetizing food cues. Furthermore, the connection between pediatric obesity and sex remains

uncertain, highlighting the need for investigating potential sex-based distinctions in future research. Finally, it will also be necessary to conduct more longitudinal neuroimaging studies in order to determine whether the observed alterations are a cause or consequence of the excess weight. The impact of obesity on the developmental trajectory of the brain in children and adolescents should also be investigated.

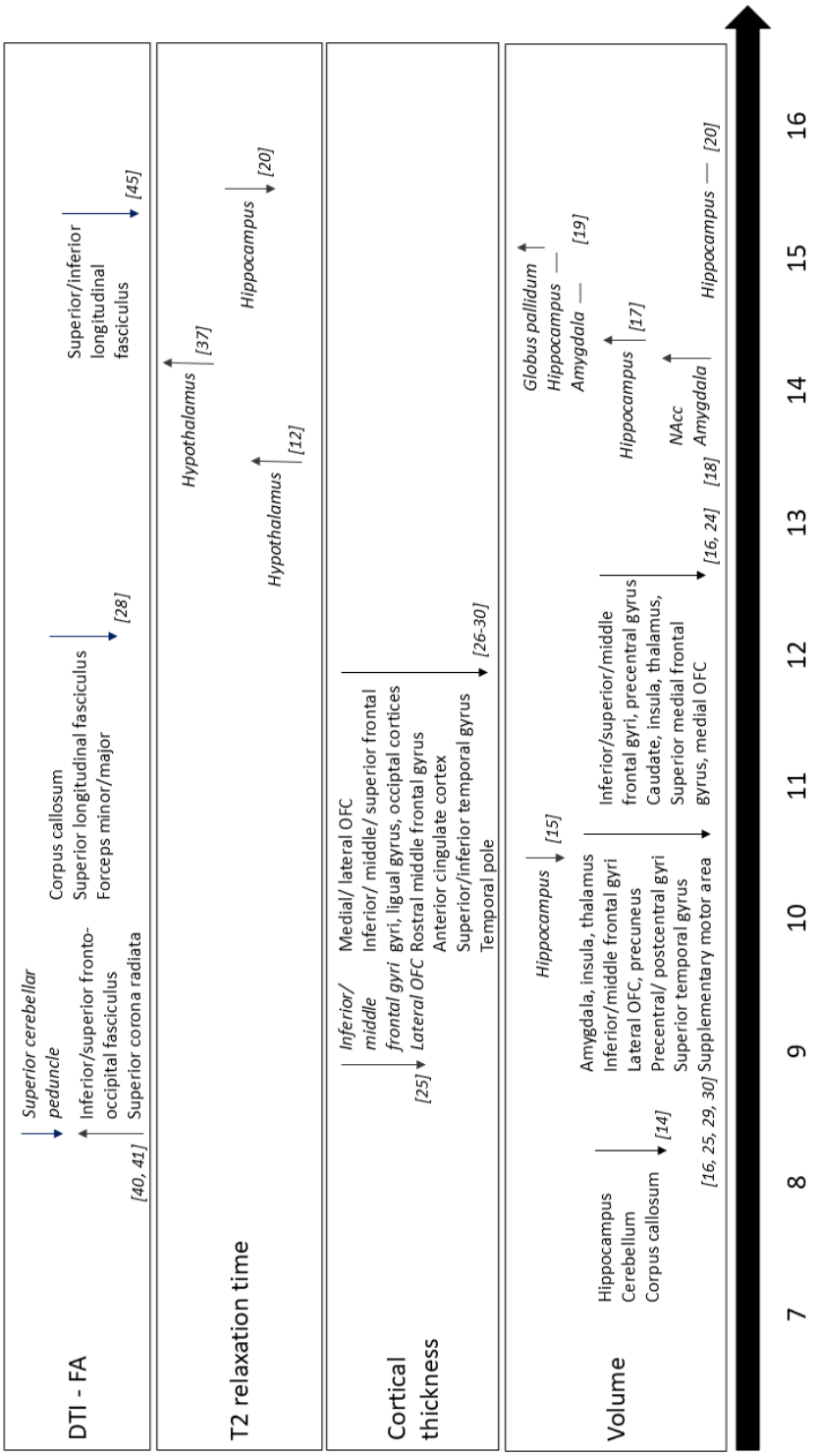

Figure 1

Structural alterations found in children and adolescents with excess weight. DTI = diffusion tensor imaging; FA = fractional anisotropy; NAcc = nucleus accumbens; PFC = prefrontal cortex. "↑" means increased metrics. "↓" means decreased metrics. " — " means no changes in metrics. Straight font means that the study used whole brain analysis. Italic font means that the study used region of interest analysis.

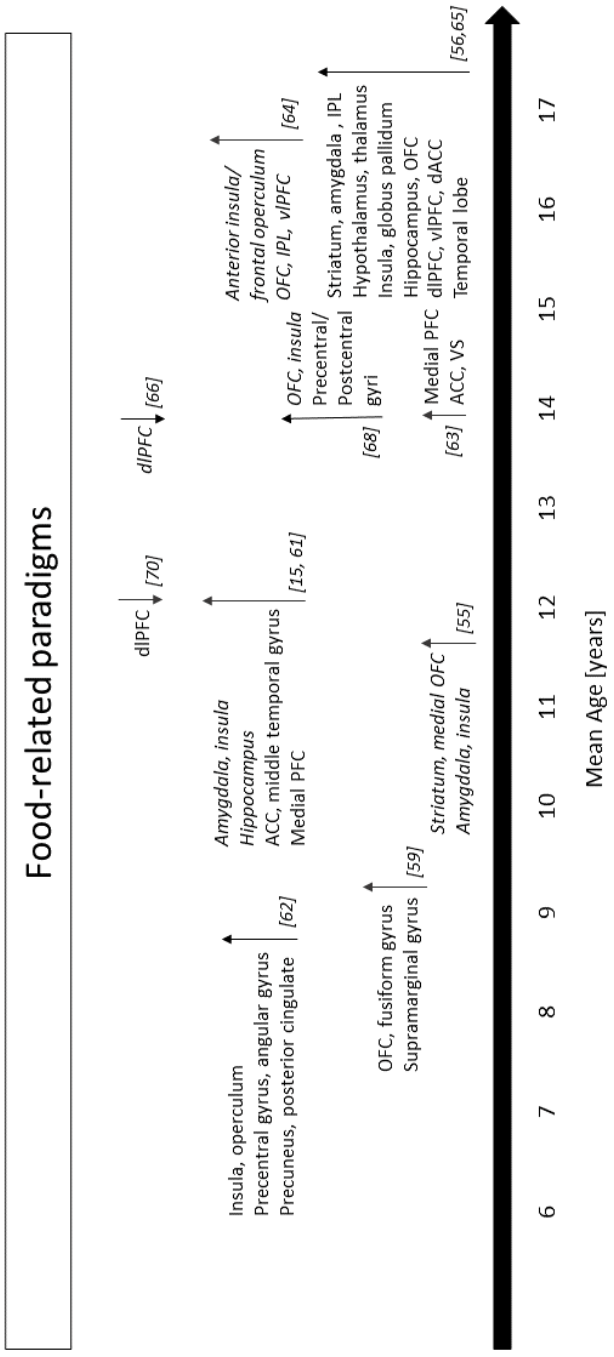

Figure 2

Functional alterations found during food-related paradigms in children and adolescents with excess weight. ACC = anterior cingulate cortex; dACC = dorsal anterior cingulate cortex; PFC = prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral prefrontal cortex; OFC = orbitofrontal cortex; IPL = inferior parietal lobe; IFG = inferior frontal gyrus. "↑" means increased activation during different paradigms. "↓" means decreased activation during different paradigms. Straight font means that the study used whole brain analysis. Italic font means that the study used region of interest analysis.

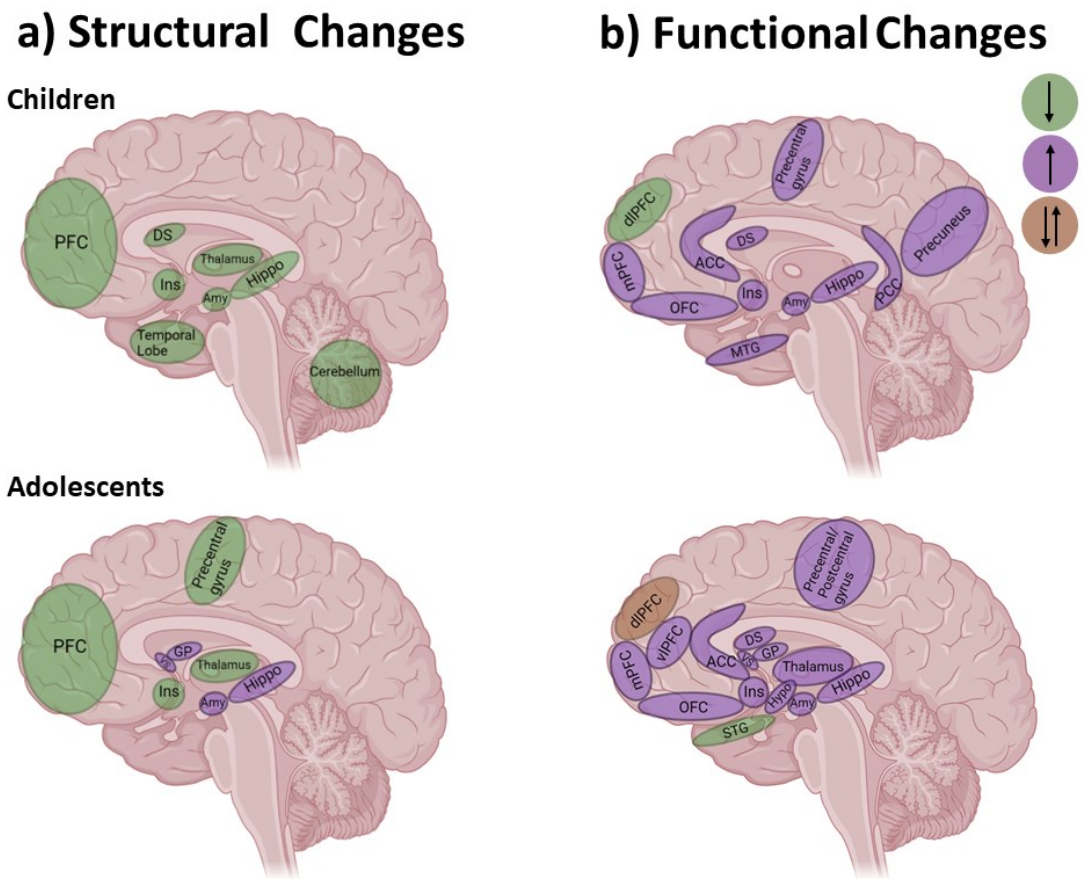

Figure 3

Altered brain structures and functions in pediatric obesity are dependent on the developmental stage of the children and adolescents. a) structural changes. b) functional changes. ACC = anterior cingulate cortex; Amy = amygdala; DS = dorsal striatum; dlPFC = dorsolateral prefrontal cortex; GP = globus pallidum; Hippo = hippocampus; Hypo = hypothalamus; Ins = insula; MTG = middle temporal gyrus; OFC = orbitofrontal cortex; PFC = prefrontal cortex; PCC = posterior cingulate cortex; VS = ventral striatum; vlPFC = ventrolateral prefrontal cortex; STG = superior temporal gyrus. Figure was created with BioRender.com.

Table 1

Description of structural MRI and DTI studies in children and adolescents with excess weight.

Author	Study design	Study sample	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI% (M, SD)	Gender (% female)	Imaging modality	Metrics	Analysis methods	Main outcomes
Bauer et al. 2015 [14]	C.S.	33 (18 HW, 15 OW/ OB)	All: 7.6 (0.42)	N.S.	BMI: HW: 15.29 (1.3); OW/OB: 21.61 (5.0)	64	Structural MRI	Volume	Whole-brain analysis	Children with OW/OB: **Hippocampus ↓, cerebellum ↓, corpus callosum ↓**
Mestre et al. 2017 [15]	C.S.	25 (13 HW, 12 OB)	HW: 10.38 (1.26); OB: 10.08 (1.00)	N.S.	BMI: HW: 17.71 (1.9); OB: 26.10 (3.23)	40	Structural MRI	Volume	ROI analysis	Children with OB: **Hippocampus ↓**
Jiang et al. 2023 [16]	L.S.	523 (265 HW, 258 OB)	Month pre HW: 119.1 (0.4); OB:119.5 (0.5); post HW: 142.8 (0.4); OB: 143.2 (0.5)	N.S.	BMI: pre HW: 16.8 (0.1); OB: 26.5 (0.2); post HW: 18.1 (0.1); OB: 30.0 (0.3)	48	Structural MRI	Grey matter volume	Whole-brain analysis	Baseline children with OB: **Amygdala ↓, inferior frontal gyrus ↓, superior temporal gyrus ↓, insula ↓, thalamus ↓, supplementary motor area ↓** 2- year follow-up children with OB: **inferior/superior/middle frontal gyrus ↓, superior medial frontal gyrus ↓, precentral gyrus ↓, caudate ↓, thalamus ↓, amygdala ↓**
Moreno-Lo´pez et al. 2012 [17]	C.S.	52 (16 HW, 36 OW/OB)	HW: 14.13 (1.36); OW/OB: 14.22 (1.4)	N.S.	BMI: HW: 20.26 (2.8); OW: 24.85 (1.42); OB: 31.46 (2.91)	67	Structural MRI	Volume	ROI/ Whole-brain analysis	Adolescents with OW/OB: **Hippocampus ↑**

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Author	Study design	Study sample	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI% (M, SD)	Gender (% female)	Imaging modality	Metrics	Analysis methods	Main outcomes
Perlaki et al. 2018 [18]	C.S.	51 (in total)	All: 13.8 (1.9)	2-5	BMIz: All: 0.38 (1.24)	63	Structural MRI	Volume	ROI analysis	BMI z-score ↑, **NAcc ↑**, **amygdala ↑**
deGroot et al.2017 [19]	C.S.	42 (19 HW, 23 OB)	12-16	N.S.	N.S.	N.S.	Structural MRI	Volume	ROI analysis	Adolescents with OB: **Globus pallidum ↑** No relationship between BMI and the volume of striatum, hippocampus, amygdala
Hashimoto et al. 2015 [21]	L.S.	107 (in total)	pre: 11.1; post: 14.1	N.S.	BMI: pre:13.2-27.4; post: 12.5-37.1	47	Structural MRI	Volume	Whole-brain analysis	Adolescents: During 3-years development, BMI increase ↓, the increase of the volume in the posterior **hippocampus ↑**, **parahippocampal gyrus ↑**
Migueles et al. 2021 [22]	C.S.	96 (in total)	All: 10.02 (1.13)	N.S.	BMIz: All: 3.04 (0.89)	40	Structural MRI	Volume	ROI/ Whole-brain analysis	Children with OW/OB: Total sleep time ↑, sleep efficiency ↑, wakening after sleep onset ↓, **hippocampus↑**; Sleep behaviors were associated with higher volume in several cortical brain regions
Higgins et al. 2022 [23]	C.S.	149 (in total)	All: 8.99 (1.21)	N.S.	BMI%: All: 98.2 (17.5)	51	Structural MRI	Grey matter volume	ROI analysis	Exclusive breastfeeding duration ↑, **hippocampus ↑**
Zhang et al. 2023 [24]	C.S.	476 (244 HW, 232 OW/ OB)	HW: 144.34 (0.46); OW/OB: 144.16 (0.51)	N.S.	BMI: HW: 18.16 (0.11); OW/OB: 27.07 (0.29)	45	Structural MRI	Grey matter volume	Whole-brain analysis	Children with OW/OB: **Superior frontal gyrus ↓**, **dorsal anterior cingulate cortex ↓**, **medial superior frontal gyrus ↓**, **medial orbital frontal cortex ↓**

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Author	Study design	Study sample	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI% (M, SD)	Gender (% female)	Imaging modality	Metrics	Analysis methods	Main outcomes
Hall et al. 2023 [25]	L.S.	11226 (in total)	Month pre all: 119.1 (7.5); post all: 131.2 (7.7)	1-5	BMIz: pre all: 0.9 (2.2); post all: 1.4 (2.4)	48	Structural MRI	Grey matter volume/ cortical thickness	ROI analysis	BMI z-score ↑, **inferior/ middle frontal gyrus ↓, lateral orbitofrontal cortex ↓** 1-year follow-up: increases in BMI z-score ↑, **middle frontal gyrus ↓**
Laurent et al. 2020 [26]	C.S.	3190 (in total)	ALL: month 120.2 (7.3)	1-5	BMI: All: 18.64 (3.9)	49	Structural MRI	Cortical thickness	Whole-brain analysis	BMI ↑, **OFC ↓, pars triangularis ↓, frontal pole ↓, rostral middle frontal gyrus ↓, superior frontal gyrus ↓, superior/inferior temporal gyrus ↓, temporal pole ↓**
Ronan et al. 2020 [27]	C.S.	2700 (in total)	9-11	N.S.	N.S.	50	Structural MRI	Cortical thickness	Whole-brain analysis	At baseline and second year: BMI ↑, **OFC ↓, inferior frontal gyrus ↓, rostral middle frontal gyrus ↓, superior frontal gyrus ↓, temporal pole ↓**
Kaltenhauser et al. 2023 [28]	L.S.	4576 (in total)	ALL: month 119.8 (7.6)	1.5 (0.5)	BMIz: All: 0.3 (1.1)	48	Structural MRI + DTI + fMRI	Cortical thickness/ FA/ rs-FC	Whole-brain analysis	BMI ↑, waist circumference ↑, cortical thickness in the **rostral middle frontal gyrus ↓**; FA of the **corpus callosum**, **superior longitudinal fasciculus**, **forceps minor/major ↓**; FC within **salience network ↓** ; BMI at baseline ↑, FA in the **inferior-fronto-occipital fasciculi**, **anterior thalamic radiations**, **corpus callosum** in 2-years ↓

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Author	Study design	Study sample	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI% (M, SD)	Gender (% female)	Imaging modality	Metrics	Analysis methods	Main outcomes
Brooks et al. 2023 [29]	C.S.	4922 (in total)	ALL: month 120.0 (13.0)	1-4	BMIz: HW: -0.46 (0.57); OW: 0.66 (0.40); OB: 1.75 (0.97)	52	Structural MRI + fMRI	Cortical thickness/ volume/ rs-FC	Whole-brain analysis	Children with OW/OB: **Superior/ middle frontal gyrus ↓, anterior cingulate cortex ↓, medial/ lateral OFC ↓, lateral occipital cortices ↓**; FC, topological efficiency, resilience, connectedness, clustering in **default mode network ↓, dorsal attention network ↓, salience network ↓, executive control network ↓**
Cui et al. 2023 [30]	C.S.	8484 (HW: 5759; OW: 1302 OB: 1423)	Month HW: 118.96 (0.10); OW: 119.14 (0.21) OB: 118.62 (0.20)	N.S.	BMI: HW: 16.73 (0.02); OW: 20.83 (0.03); OB: 26.13 (0.10)	58	Structural MRI	Cortical thickness/ volume	Whole-brain analysis	BMI ↑, cortical thickness in the **lateral OFC ↓, lingual gyrus ↓**, cortical volume in the precentral gyrus ↓, postcentral gyrus ↓, **precuneus ↓, superior parietal lobule ↓, insula ↓**
Gracia-Marco et al. 2020 [31]	C.S.	100 (in total)	All: 10.0 (1.1)	N.S.	BMI: All: 26.7 (3.7)	40	Structural MRI	White/ grey matter volume	Whole-brain analysis	Children with OW/OB: Lean mass index ↑, white matter **putamen ↑, superior frontal gyrus ↑, superior fronto-medial gyrus ↑, middle fronto-orbital gyrus ↑, cerebellum ↑, parietal region ↑** lean mass index ↑, grey matter **superior fronto-orbital gyrus ↑**
Sakib et al. 2023 [32]	L.S.	11103 (in total)	All: 9.91 (0.6)	Pre: 1.7 (0.8) Post: 2.5 (1.0)	BMIz: pre all: 1.0 (2.4); post all: 1.9 (2.4)	48	Structural MRI	Grey matter volume/ cortical thickness	ROI analysis	The volume and cortical thickness of **middle frontal gyrus** mediated the inverse association between executive function and BMI z-score

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Author	Study design	Study sample	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI% (M, SD)	Gender (% female)	Imaging modality	Metrics	Analysis methods	Main outcomes
Saute et al. 2018 [33]	C.S.	44 (26 HW, 18 OB)	HW: 16.81 (0.71); OB: 16.22 (0.73)	N.S.	BMI: HW: 21.38 (1.70); OB: 31.11 (3.21)	50	Structural MRI	Cortical thickness	Whole-brain analysis	Visceral abdominal fat ↑, **superior frontal gyrus** ↑, **superior temporal gyrus** ↑, **pre/postcentral gyrus** ↑, **lateral occipital gyrus** ↑, **precuneus** ↑
Adelantado-Renau et al. 2019 [34]	C.S.	107 (in total)	All: 10.0 (1.1)	N.S.	BMI: All: 26.7 (3.7)	41	Structural MRI	Volume	Whole-brain analysis	C-reactive protein ↑, **superior frontal gyrus** ↓; interleukin-6 ↑, tumor necrosis factor-α ↑, **inferior frontal gyrus** ↑; white blood cell ↑, **middle temporal gyrus** ↑; C-reactive protein ↑, **superior temporal gyrus** ↑
Solis-Urra et al. 2019 [35]	C.S.	96 (in total)	All: 10.0 (1.1)	N.S.	BMI: All: 26.7 (3.6)	38	Structural MRI	Gray matter volume	Whole-brain analysis	Higher birth weight and birth length ↑, prolonged breastfeeding ↑, volume of regions involved in higher order cognition and emotion regulation ↑
Sewaybricker et al. 2019 [12]	C.S.	23 (12 HW, 11 OB)	HW: 13.3 (2.1); OB: 12.9 (2.7)	N.S.	BMIz: HW: 0.4 (0.8); OB: 2.1 (0.3)	48	Structural MRI	T2 relaxation time	ROI analysis	Children with OB: **hypothalamus** ↑
Sewaybricker et al. 2021 [36]	C.S.	31 (20 HW, 11 OW/ OB)	All: 13.8 (2.5)	N.S.	BMIz: All: 0.75 (1.10)	61	Structural MRI	T2 relaxation time	ROI analysis	BMI z-score ↑, **mediobasal hypothalamus** ↑
Sewaybricker et al. 2021 [37]	L.S.	238 (114 OW, 124 OB)	OW: 9.9 (0.6) OB: 9.9 (0.6)	1-5	BMz: HW: 1.37 (0.17); OB: 2.04 (0.26)	47	Structural MRI	T2 relaxation time	ROI analysis	Children with OW, but not OB: **Hypothalamus/amygdala** ↑, adiposity gain over one year ↑

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Author	Study design	Study sample	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI% (M, SD)	Gender (% female)	Imaging modality	Metrics	Analysis methods	Main outcomes
Mestre et al. 2020 [20]	C.S.	102 (in total)	All: 15.07 (1.84)	N.S.	BMIz: All: 0.54 (1.17)	53	Structural MRI	T2 relaxation time + Volume	ROI analysis	BMI z-score ↑, T2 signal intensity in the **hippocampus** ↓ no relationship between BMI z-score and hippocampal volume
Ou et al. 2015 [40]	C.S.	24 (12 HW, 12 OB)	HW: 9.8 (0.7); OB: 9.1 (0.9)	N.S.	BMI: HW: 15.8 (1.0); OB: 24.4 (3.4)	50	Structure +DTI	FA	Whole-brain analysis	Children with OB: Posterior part of the **inferior/superior fronto-occipital fasciculus** ↑, **superior corona radiata** ↑
Augustijn et al. 2018 [41]	C.S.	44 (25 HW, 19 OB)	HW: 9.5 (1.2); OB: 9.4 (1.0)	N.S.	BMI: HW: 16.90 (1.15); OB: 31.03 (4.62)	34	DTI	FA	ROI analysis	Children with OB: **Superior cerebellar peduncle** ↓, motor competence ↓
Augustijn et al. 2019 [42]	L.S.	40 (22 HW, 18 OB)	pre HW: 9.6 (1.2); OB: 9.5 (1.0); post HW: 10.0 (1.2); OB: 9.9 (1.0)	1-3	BMI: pre HW: 16.85 (1.15); OB: 31.64 (4.35); post HW: 16.93 (1.19); OB: 25.66 (3.68)	25	DTI	Network connected strength	graph-theoretical approach and network-based statistics	Children with OB: Structural connected strength between **putamen** and **caudate**, **NAcc** ↑; Multicomponent behavioral intervention was not associated with alterations in the brain networks

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Author	Study design	Study sample	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI% (M, SD)	Gender (% female)	Imaging modality	Metrics	Analysis methods	Main outcomes
Solis-Urra et al. 2022 [43]	C.S.	98 (in total)	All: 10.03 (1.16)	N.S.	BMI: All: 26.58 (3.64)	39	DTI	FA/MD	Whole-brain analysis	Head circumference at birth ↑, FA in the **inferior fronto-occipital fasciculus** ↑, MD in the **cingulate gyrus part of cingulum** ↓, **corticospinal and superior thalamic radiation** ↓
Li et al. 2023 [44]	C.S.	8842 (in total)	All: month 119 (8)	1-4	BMI-z: All: 0.4 (1.2)	49	DTI	RSI-RND RSI-RNI		Obesity mediates negative relationships between household socioeconomic status and maturity maturation of the **inferior longitudinal fasciculus**, **anterior thalamic radiations**, **forceps major**
Alarcón et al. 2016 [45]	C.S.	152 (88 HW, 64 OW/ OB)	HW: 14.2 (0.1); OW:13.8 (0.2) OB: 14.4 (0.4)	HW: 3.5 (0.1); OW: 3.5 (0.1); OB: 3.4 (0.2)	BMI%: HW: 58.9 (1.8); OW: 90.0 (0.4); OB: 96.9 (0.3)	43	DTI	FA	Whole-brain analysis	BMI ↑, **superior/inferior longitudinal fasciculus** ↓

Note. " ↑ " means increased metrics; " ↓ " means decreased metrics; BMI = body mass index; BMIz = BMI z-score; C.S.= cross-sectional study; DTI = diffusion tensor imaging; M = mean; fMRI = functional MRI; FA = fractional anisotropy; HW = healthy weight; L.S.= longitudinal study; MRI = magnetic resonance imaging; MD = mean diffusivity; N.S. = not specified; NAcc = nucleus accumbens; OW = overweight; OB = obesity; OFC = orbital frontal cortex; rs-fMRI = resting state fMRI; ROI = region of interest; ROI: region of interest; RSI = restriction spectrum imaging; RND = restricted normalized directional diffusion; RNI = restricted normalized isotropic diffusion; SD = standard deviation.

Table 2
Description of fMRI studies in children and adolescents with excess weight.

Author	Study design	Sample size	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI % (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Borowitz et al. 2020 [47]	C.S.	164 (88 HW, 76 OW/ OB)	HW: 14.14 (1.04); OW: 14.14 (1.01); OB: 14.58 (0.97)	N.S.	BMIz: HW: 0.14 (0.61); OW: 1.35 (0.17); OB: 2.06 (0.32)	53	fMRI	Resting state		ROI analysis	Adolescent obesity ↑, ↑ FC between the **medial OFC** and the **globus pallidum, olfactory tubercle**; ↓ FC between the **medial OFC** and the **ventrolateral PFC**; ↓ FC between the **hippocampus** and the **caudate**
Martín-Pérez et al. 2019 [48]	C.S.	104 (51 HW, 53 OW/ OB)	HW: 15.29 (1.75); OW/OB : 14.64 (1.78)	N.S.	BMI%: HW: 52.35 (24.35); OW/OB: 93.98 (3.98)	65	fMRI	Resting state		ROI analysis	Adolescents with OW/OB: ↑ FC between the **hypothalamus** and the **OFC**, **VS**, **anterior insula**, **middle temporal cortex**; ↓ FC between the **hypothalamus** and the **cerebellum**, **middle prefrontal**, **precentral/ postcentral gyri**
Moreno-Lopez et al. 2016 [49]	C.S.	115 (55 HW, 60 OW/ OB)	HW: 15.11 (1.82); OW/OB : 14.67 (1.70)	N.S.	BMI: HW: 20.84 (2.39); OW/OB: 29.26 (3.84)	61	fMRI	Resting state		ROI/ Whole-brain analysis	Adolescents with OW/OB: ↑ FC between the **middle temporal gyrus** and the **OFC;** ↓ FC between the **insula** and the **ACC**; ↓ FC between the **middle temporal gyrus** and the **PCC**
Black et al. 2014 [50]	C.S.	18 (9 HW, 9 OB)	HW: 12.3 (1.41); OB: 11.66 (0.87)	N.S.	BMI%: HW: 51.33 (20.43); OB: 97.80 (1.81)	56	fMRI	Resting state		ROI analysis	Children with OB: ↑ FC between the **middle frontal gyrus** and the **ventromedial PFC** and the **lateral OFC**

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Author	Study design	Sample size	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI % (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Pujol et al. 2021 [51]	C.S.	230 (147 HW, 83 OW/ OB)	All: 9.8 (0.9)	N.S.	BMI: All: 18.0 (2.8)	50	fMRI	Resting state		ROI analysis	Children with OW/OB: ↓ FC between the **OFC** and the **NAcc, amygdala**
Solis-Urra et al. 2022 [54]	C.S.	96 (in total)	All: 10.01 (1.14)	N.S.	BMI: All: 26.7 (3.69)	38	fMRI	Resting state		ROI analysis	Birth weight ↑, ↑ FC between the **hippocampus** and the **pre/postcentral gyri**, **cerebellum**; breastfeeding ↑, ↑ FC between the **hippocampus and** the **middle temporal gyrus** ↓, FC between the **hippocampus** and the **primary motor cortex**, **angular gyrus**
Roth et al. 2019 [55]	C.S.	76 (22 HW, 54 OB)	HW: 10.4 (0.9); OB: 10.4 (0.8)	N.S.	BMI%: HW: 46 (18); OB: 98 (1.1)	45	fMRI	Visual food cue task	1st fMRI: eat 3 h prior fMRI; 2nd fMRI: after a test meal	ROI/ Whole-brain analysis	Children with OB: Activation post-meal to high-calorie food stimuli (vs. objects) in the **medial OFC** ↑, **VS** ↑, **DS** ↑, **amygdala** ↑, **substantia nigra/ventral tegmental area** ↑, **insula** ↑, despite normal ghrelin responses
Jastreboff et al. 2014 [56]	C.S.	40 (15 HW, 25 OB)	HW: 15.5 (1.38); OB: 15.69 (1.77)	HW: 4.6; OB: 4.2	BMI-z: HW: 0.21 (0.46); OW/OB: 2.19 (0.34)	50	fMRI	Visual food cue task	Eat 2 h prior fMRI	Whole-brain analysis	Adolescents with OB: Response to high-calorie food (vs. nonfood) in the **striatal-limbic regions** ↑; All subjects: Leptin ↑, activation in the **motivation-reward regions** ↑

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Author	Study design	Sample size	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI % (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Adam et al. 2015 [57]	C.S.	12 (12 OW)	OW: 9.9 (1.1)	OW: 1-2	BMI: OW: 29.9 (5.7)	100	fMRI	Visual food cue task	Eat 3 h prior fMRI	Whole-brain analysis	Girls with OW: Insulin sensitivity ↑, activation in the **ACC ↓**, **OFC ↓**, **insula ↓**, **frontal operculum ↓**, **rolandic operculum ↓** to high calorie food (vs. non-food)
Yokum et al. 2013 [58]	C.S.	21 (in total)	All: 15.2 (1.18)	N.S.	BMI: All: 27.9 (5.16)	62	fMRI	Cognitive reappraisal strategies + visual food cue task	Eat 5 h prior fMRI	Whole-brain analysis	Activation in the **superior/middle frontal gyrus ↑**, **ventrolateral prefrontal cortex ↑**, **posterior cingulate cortex ↓**, **precuneus ↓** during food stimuli with cognitive reappraisal strategies
Masterson et al. 2019 [59]	C.S.	41 (25 HW, 16 OW/ OB)	HW: 7.84 (0.68); OW/OB: 8.00 (0.73)	N.S.	BMI%: HW: 48.00 (18.00); OW/OB: 91.00 (5.00)	54	fMRI	food cue task after food/toy commercial exposure	Eat 3 h prior fMRI	Whole-brain analysis	Children with excess weight: brain response to high-calorie food stimuli in the **OFC ↑**, **fusiform gyrus ↑**, **supramarginal gyrus ↑**
Jensen et al. 2019 [60]	C.S.	52 (29 HW, 23 OW/ OB)	All: 15.96 (1.56)	N.S.	BMI%: HW: 54.55 (24.54); OW/OB: 93.78 (4.60)	N.S.	fMRI	Sleep restriction, food specific go/no-go task	4h	ROI/ Whole-brain analysis	Adolescents with NW, but not OW: Sleep restriction ↑, brain response to food cues in the **middle/inferior frontal gyrus ↑**, **ACC↑**
Mestre et al. 2017 [15]	C.S.	25 (13 HW, 12 OB)	HW: 10.38 (1.26); OB: 10.08 (1.00)	N.S.	BMI: HW: 17.71 (1.9); OB: 26.10 (3.23)	40	fMRI	Tasting	Sated	ROI analysis	Children with OB: Activation to taste in the **hippocampus ↑**

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Author	Study design	Sample size	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI % (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Boutelle et al. 2015 [61]	C.S.	23 (13 HW, 10 OB)	HW: 10.4 (0.3); OB: 9.9 (0.3)	N.S.	BMI%: HW: 53.9 (6.9); OB: 96.8 (0.5)	44	fMRI	Tasting	Sated	ROI/ Whole-brain analysis	Children with OB: Response to taste in the **amygdala, insula, medial frontal cortex**↑
Bohon et al. 2017 [62]	C.S.	18 (10 HW, 8 OW)	6-8	N.S.	BMI%: HW: 5-73; OW: 88-95	72	fMRI	Tasting, visual food cue task	Eat 4-6 h prior MRI	Whole-brain analysis	Children with OW: Response to taste in the **insula ↑, operculum ↑, precentral gyrus ↑, angular gyrus ↑, precuneus ↑ and posterior cingulate ↑**; no differences for visual food cues
Yokum et al. 2023 [63]	C.S.	88 (10 HW, 8 OW)	HW: 14.6 (0.93); OW/OB :14.5(0.84)	N.S.	BMI: HW: 20.3 (2.0); OW/OB: 27.0 (2.8)	100	fMRI	Tasting	Eat 3-4 h prior MRI	Whole-brain analysis	Adolescents with OW/OB: Response to taste in the **medial frontal cortex**↑, **ventral anterior cingulate cortex ↑**, **VS ↑**
Yokum et al. 2011 [64]	L.S.	39 (in total)	All: 15.6 (0.96)	N.S.	BMI: All: 24.2 (4.5)	100	fMRI	Food specific attention network task	Eat 4-6 h prior MRI	ROI analysis	BMI ↑, activation in the **anterior insula/frontal operculum ↑**, during reallocation of attention to appetizing food cues Activation in the **OFC** predicted weight gain one-year later
Moreno-Padilla et al. 2018 [65]	C.S.	77 (39 HW, 38 OW/ OB)	HW: 16.58 (1.63); OW/OB : 16.47 (1.66)	N.S.	BMI: HW: 21.36 (2.07); OW/OB: 29.89 (3.72)	52	fMRI	Food choice task	Eat 1-3 h prior MRI	Whole-brain analysis	Adolescents with OW/OB: Activation in the **striatum, OFC, globus pallidum, insula, hippocampus, dACC, dlPFC, ventrolateral PFC ↑**, during choosing appetizing food (vs. plain food)

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Author	Study design	Sample size	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI % (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
van Meer et al. 2019 [66]	L.S.	141 (in total)	All: 13.4 (1.8)	All: 2.43 (0.59)	BMI cole score: All 0.48 (1.04)	57	fMRI	Food choice task	Eat 2 h prior fMRI	ROI analysis	Children and adolescents: Age ↓with BMI ↑, activation in the **dlPFC** ↓, during food choice Higher response in the attentional regions predicted greater weight gain per year
Lim et al. 2023 [67]	C.S	141 (in total)	All: 15.88 (0.93)	N.S.	BMI%: All: 71.98 (25.22)	0	fMRI	Food and physical activity decision task	4 h	Whole-brain analysis	Adolescents with OW/OB: Activations in the **inferior frontal cortex** ↓, **motor cortex** ↓, and **superior temporal gyrus** ↓ during physical activity decisions
Rapuano et al. 2016 [68]	C.S.	37 (19 HW, 18 OW/ OB)	All: 14.4 (1.3)	N.S.	BMI: HW: 20.15 (2.05); OW/OB: 33.20 (2.51)	54	fMRI	Food/non-food commercial	Eat 2 h prior fMRI	ROI/ Whole-brain analysis	Percent of body fat ↑, activation in the **OFC** ↑, **insula** ↑, **mouth-specific somatosensory-motor cortices** ↑ to food commercial
Burger et al. 2014 [69]	C.S.	25 (in total)	All: 15.2 (0.8)	N.S.	BMI%: All: 67.4 (22.5)	48	fMRI	Food/non-food commercial	4h	Whole-brain analysis	Soft drink commercial activated the **insula**, **putamen**, **postcentral gyrus** ↑
Bruce et al. 2013 [70]	C.S.	20 (10 HW, 10 OB)	All: 11.85 (1.23)	N.S.	BMI%: HW: 50 (19.7); OB: 98.9 (1.7)	55	fMRI	Food/non-food logo	Min. 4 h	Whole-brain analysis	Children with OB: Activation to food logos in the **middle/inferior prefrontal cortex** ↓
Gearhardt et al. 2020 [71]	C.S.	171 (in total)	13-16	N.S.	N.S.	N.S.	fMRI	Fast food/non-food commercial	N.S.	ROI/ Whole-brain analysis	Activation in the **NAcc** ↑, **caudate** ↑, **hippocampus** ↑ during fast food commercials, eating in the laboratory ↑
Yokum et al. 2014 [72]	L.S.	40 (in total)	All: 15.2 (1.1)	N.S.	BMI: All: 26.9 (5.4)	57	fMRI	Food/non-food commercial	5h	ROI analysis	Activation in the **striatum** ↑to food (vs. non-food commercial), BMI increases one-year after↑

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Author	Study design	Sample size	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI % (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Navas et al. 2018 [73]	C.S.	68 (in total)	All: 16.56 (1.35)	N.S.	BMI%: All: 69.33 (28.83)	50	fMRI	Monetary Incentive Delay task	N.S.	ROI analysis	Adiposity ↑, activation in the **somatosensory regions** ↓, during general reward feedback
Adise et al. 2019 [74]	C.S.	61 (30 HW, 31 OW/ OB)	HW: 8.7 (1.4); OW/OB: 9.4 (1.2)	HW: 1.6 (0.8); OW/OB: 1.9 (1.1)	BMI%: HW: 53.4 (11.8); OW/OB: 94.5 (4.0)	54	fMRI	Modified card-guessing task	Min. 3 h	ROI/ Whole-brain analysis	All children: Response to winning money (vs. food) in the **striatum** ↑, regardless of weight status
Adise et al. 2018 [75]	C.S.	59 (31 HW, 28 OW/ OB)	HW: 8.7 (1.4); OW/OB: 9.4 (1.2)	HW: 1.6 (0.8); OW/OB: 1.9 (1.1)	BMI%: HW: 53.4 (11.8); OW/OB: 94.5 (4.0)	54	fMRI	Modified card-guessing task	Min. 3 h	Whole-brain analysis	Brain response to food (vs. money) rewards in the **amygdala ↑, OFC ↑, medial PFC** ↑, overeating ↑, independent of weight status
Delgado-Rico et al. 2013 [76]	C.S.	52 (16 HW, 36 OW/ OB)	HW: 13.88 (1.36); OW: 14.07 (1.67); OB: 14.29 (1.31)	N.S.	BMI: HW: 20.19 (2.80); OW: 24.65 (1.26); OB: 31.33 (2.92)	50	fMRI	Risky-Gains task	N.S.	Whole-brain analysis	Adolescents with OW/OB: Activation in the **insula** ↓, **midbrain** ↑, during risky vs. safe choices; activation in the **inferior frontal gyrus** ↑, **thalamus** ↑, **parahippocampus** ↑, **cerebellum** ↑, during reward vs. punishment feedback
Mata et al. 2015 [77]	C.S.	54 (32 HW, 22 OW/ OB)	HW: 15.53 (1.70); OW/OB: 15.14 (2.03)	N.S.	BMI: HW: 21.17 (2.24); OW/OB: 29.40 (3.00)	61	fMRI	Risky-Gains task	N.S.	ROI analysis	Activation in the **insula** ↑, Interoceptive sensitivity ↓, restrained eating ↓, external eating ↑

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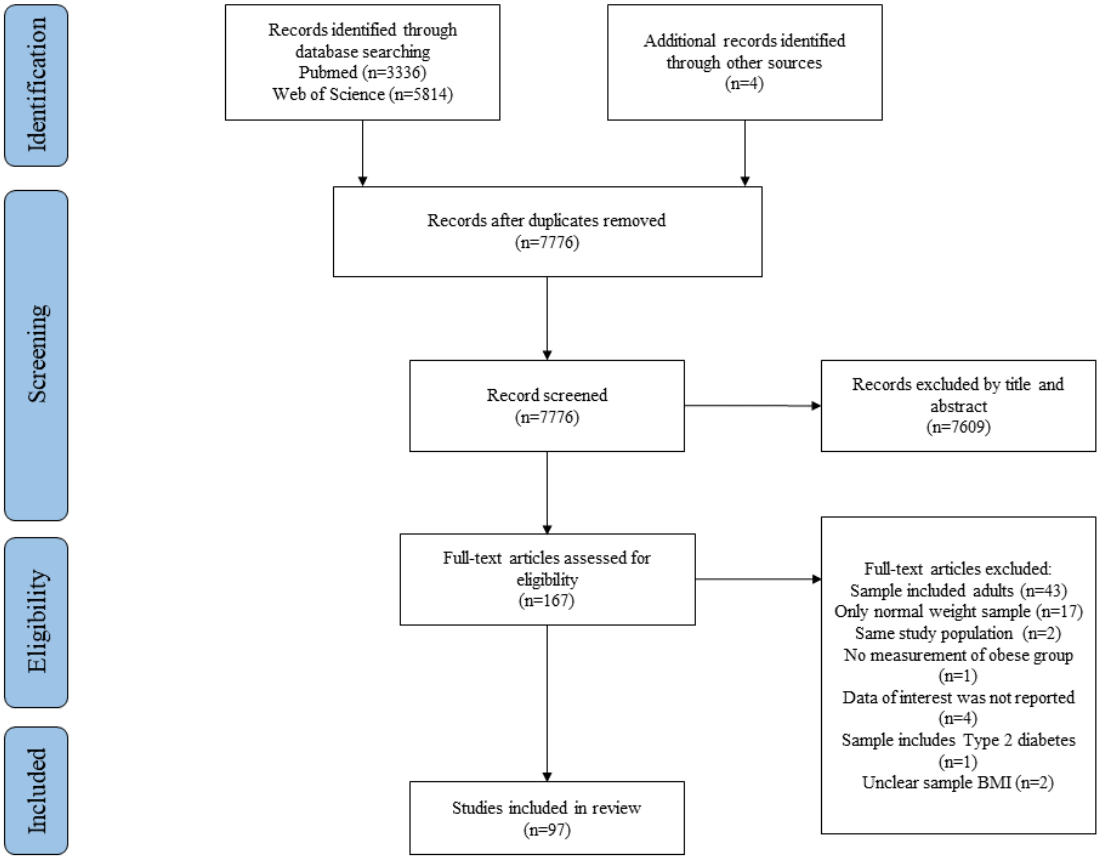
Author	Study design	Sample size	Age (M, SD)/ age range	Tanner stage (M)/ range	Weight status: BMIz/ BMI/ BMI cole score/ BMI % (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Verdejo-García et al. 2015 [78]	C.S.	80 (44 HW, 36 OW/ OB)	HW: 15.32 (1.69); OW/OB: 15.06 (1.88)	N.S.	BMI: HW: 20.96 (2.31); OW/OB: 29.11 (3.90)	61	fMRI	Social decision-making task (ultimatum game)	N.S.	Whole-brain analysis	Adolescents with OW/OB: Activation in the **ACC** ↓, **anterior insula** ↓, **thalamus** ↓, **midbrain** ↓, during decisions about unfair (vs. fair) offers
Jensen et al. 2022 [79]	C.S.	42 (in total)	All: 16.48 (1.01)	N.S.	BMI%: All 94.57 (4.4)	100	fMRI	Sleep deprivation, social stress induction task	4h	ROI analysis	OW adolescent females with sleep deprivation: Sleep deprived ↑, activation in the **putamen** ↑, **hippocampus** ↑ during social evaluation; Activation in the **ACC** during negative social feedback ↑, calorie consumption ↑

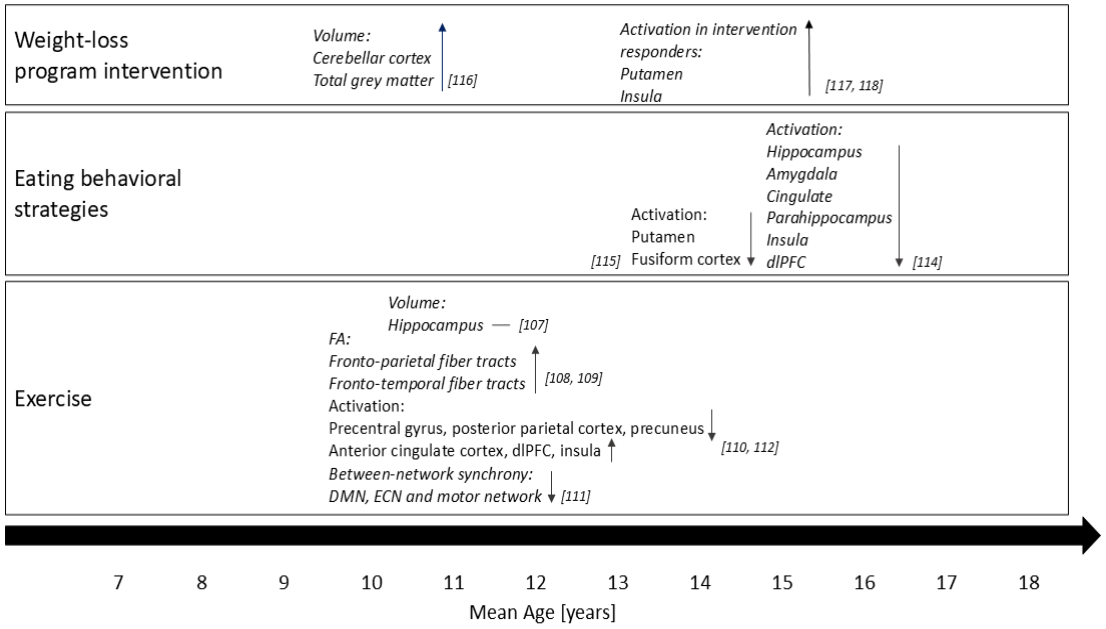
Note. " ↑ " means increased activation during different paradigms or increased functional connectivity; " ↓ " means decreased activation during different paradigms or decreased functional connectivity; ACC = anterior cingulate cortex; BMI = body mass index; BMIz = BMI z-score; C.S.= cross-sectional study; DS = dorsal striatum; dlPFC = dorsolateral prefrontal cortex; fMRI = functional MRI; FC = functional connectivity; FA = fractional anisotropy; HW = healthy weight; L.S.= longitudinal study; M = mean; MRI = magnetic resonance imaging; N.S. = not specified; NAcc = nucleus accumbens; OW = overweight; OB = obesity; OFC = orbitofrontal cortex; PFC = prefrontal cortex; PCC = posterior cingulate cortex; ROI: region of interest; rs-fMRI = resting state fMRI; SD = standard deviation; VS = ventral striatum.

Supplementary Text

Search terms for PubMed were as follows: ((neuroimaging [MeSH]) OR (magnetic resonance imaging [MeSH]) OR (diffusion tensor imaging [MeSH]) OR (neuroimaging [TW]) OR (magnetic resonance imaging [TW]) OR (fMRI [TW]) OR (functional MRI [TW]) OR (functional magnetic resonance imaging [TW]) OR (resting state [TW]) OR (functional connectivity [TW]) OR (structure [TW]) OR (structural [TW]) OR (volume [TW]) OR (diffusion tensor imaging [TW]) OR (DTI [TW]) OR (Arterial Spin Labeling [TW]) OR (ASL [TW])) AND ((child [MeSH]) OR (adolescent [MeSH]) OR (child [TW]) OR (children [TW]) OR (adolescent [TW])) AND ((obesity [MeSH]) OR (obesity [TW]) OR (obese [TW])). Search terms for Web of science were as follows: (((((((TS=(neuroimaging)) OR TS=(magnetic resonance imaging)) OR TS=(fMRI)) OR TS=(resting state)) OR TS=(functional connectivity)) OR TS=(structure)) OR TS=(volume)) AND ((((TS=(children and obesity)) OR TS=(adolescent and obesity)) OR TS=(children and obese)) OR TS=(adolescent and obese)). Additional potential studies were searched in the reference sections of eligible articles.



Supplementary Figure 1
Flow chart of study selection process.



Supplementary Figure 2
Brain changes after diverse interventions in children and adolescents with excess weight.

FA = fractional anisotropy; ECN = executive control network; DMN = default mode network; dlPFC = dorsolateral prefrontal cortex. “↑” means increased activation during different paradigms. “↓” means decreased activation during different paradigms or decreased functional connectivity. “ — ” means no changes in metrics. “Exercise” section includes aerobic and resistance exercise. “Eating behavioral strategies” section includes 1) having breakfast instead of skipping it, 2) using food intake reduction device training, which reduces portion size and eating speed by feedback technique. “Weight-loss program intervention” section includes interventions combining exercise with dietary restriction, cognitive behavioral therapy, family management. Activation means brain response to visual food cue task, risky-gains task, antisaccade task requiring participants to view the mirror orientation of the displayed image; and flanker task, which requires participants to identify the orientation of the central symbol and press a button using the corresponding hand. Straight font means that the study used whole brain analysis. Italic font means that the study used region of interest analysis.

Supplementary Table 1
Description of studies investigating the role of parental metabolic status on pediatric obesity.

Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI%/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Thapaliya et al. 2021 [86]	C.S.	83 (25 HR, 22 LR, 36 OW/OB)	HR: 15.9 (1.2); LR: 16.5 (1.3); OW/OB: 16.0 (1.2)	1-5	BMI%: HR: 41.4 (27.5); LR: 42.7 (25.6); OW/OB: 94.5 (4.5)	49	Structural MRI			Whole-brain analysis	Lean adolescents with high risk: showed reduced gray/white matter volume or cortical thickness in the **postcentral gyrus** ↓, **opercular cortex** ↓, **ACC** ↓, **precuneus** ↓
Carnell et al. 2017 [87]	C.S.	36 (16 HR, 10 LR, 10 OW)	HR: 15.5 (1.4); LR: 16.0 (1.9); OW/OB: 15.8 (1.8)	3-5	BMI%: HR: 53 (23); LR: 51 (23); OW/OB: 95 (4)	56	fMRI	food/ nonfood words	N.S.	Whole-brain analysis	Lean adolescents with high risk: activation in the regulatory system including **dlPFC** ↓, **dACC** ↓, in response to food (vs. non-food) words
Luo et al. 2021 [88]	C.S.	76 (in total)	All: 8.62 (1.02)	1-4	BMI%: All: 69.78 (26.19)	63	fMRI	visual food cue	12-h overnight fast	ROI analysis	Maternal current BMI ↑, food cue reactivity in **dlPFC** ↓ and **ACC** ↓, after glucose ingestion in children
Alves et al. 2020 [89]	C.S.	88 (in total)	All: 8.37 (0.89)	1-3	BMIz: All: 0.73 (1.09)	58	Structural MRI			ROI analysis	Boys, but not girls: prepregnancy BMI ↑, the volume of total **hippocampus** and **subfields** ↓

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Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI%/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Lynch et al. 2021 [90]	C.S.	117 (in total)	Unexposed: 8.74 (1.11); GDM-exposed: 8.40 (0.89)	N.S.	BMIz: Unexposed: 0.72 (0.97); GDM-exposed: 0.82 (1.16)	57	Structural MRI			ROI analysis	Children exposed to GDM: radial thickness in the left **inferior body of the hippocampus** ↓
Luo et al. 2023 [91]	C.S.	8521 (in total)	Unexposed: 9.92 (0.63); GDM-exposed: 9.92 (0.62)	1-4	N.S.	49	Structural MRI			Whole-brain analysis	Children exposed to GDM: volume in the **rostral middle frontal gyrus** ↓ and **superior temporal gyrus** ↓
Page et al. 2019 [92]	L.S.	91 (in total)	All: 8.4 (0.9)	N.S.	BMIz: All: 0.75 (1.09)	60	ASL			ROI analysis	Children exposed to maternal obesity or GDM: blood flow in the **hypothalamus** ↑ hypothalamic response ↑ , BMI in one year ↑
Luo et al. 2021 [93]	C.S.	159 (in total)	All: 8.50 (0.96)	1-4	BMIz: All: 0.80 (1.10)	60	fMRI	visual food cue	12-h overnight fast	ROI analysis	Children exposed to GDM: activation in the **OFC** ↑ response to food cue, energy intake ↑
Chandrasekaran et al. 2022 [94]	C.S.	122 (in total)	All: 8.8 (1.17)	1-4	BMIz: All: 0.9 (1.12)	57	Structural MRI			ROI analysis	Children exposed to GDM before 26 weeks' gestation: gliosis in the **mediobasal hypothalamus** ↑

Note. " ↑ " , increased; " ↓ ", decreased; ACC = anterior cingulate cortex; ASL = arterial spin labeling; BMI = body mass index; BMIz = BMI z-score; C.S.= cross-sectional study; DTI = diffusion tensor imaging; dlPFC = dorsolateral prefrontal cortex; dACC = dorsal ACC; fMRI = functional MRI; FC = functional connectivity; FA = fractional anisotropy; HW = healthy weight; HR = high risk; L.S.= longitudinal study; LR = low risk; M = mean; MRI = magnetic resonance imaging; MD = mean diffusivity; N.S. = not specified; OW = overweight; OB = obesity; OFC = orbital frontal cortex; rs-fMRI = resting state fMRI; ROI: region of interest; SD = standard deviation.

Supplementary Table 2 Description of studies examining effects of different non-pharmacological interventions on the brains of children and adolescents with excess weight.

Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI%/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Esteban-Cornejo et al. 2019 [95]	C.S.	Sample 1: 100; Sample 2: 242	Sample 1: 10.0 (1.1); Sample 2: 8.6 (0.5)	N.S.	BMI: Sample 1: 26.7 (3.7); Sample 2: HW: 16.2 (1.4); Sample 2 OW/OB: 22.5 (3.4)	48	Structural MRI			Whole-brain analysis	Children with OW/OB: cardiorespiratory fitness ↑, motor fitness ↑, and muscular fitness ↑, white matter volume in the **inferior/superior temporal gyrus** ↑, **inferior fronto-opercular gyrus** ↑ **insular cortex** ↑, **caudate** ↑, **supramarginal gyrus** ↑
Esteban-Cornejo et al. 2017 [96]	C.S.	101 (in total)	All: 10.0 (1.1)	N.S.	BMI: All: 26.8 (3.6)	40	Structural MRI			Whole-brain analysis	Children with OW/OB: cardiorespiratory fitness ↑, speed-agility ↑, grey mater volume in **frontal regions** ↑ **temporal regions** ↑, **hippocampus** ↑, **caudate** ↑
Esteban-Cornejo et al. 2019 [97]	C.S.	101 (in total)	All: 10.02 (1.14)	N.S.	BMI: All: 26.76 (3.65)	40	Structural MRI			Whole-brain analysis	Children with OW/OB: cardiorespiratory fitness ↑, speed-agility ↑, **overall cortical thickness** ↑
Haapala et al. 2024 [106]	C.S	100 (in total)	All: 10.1	N.S.	N.S.	N.S.	Structural MRI			Whole-brain analysis	Children with OW/OB: cardiorespiratory fitness ↑, t**otal grey mater volume** ↑, executive functions ↑

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Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI%/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Esteban-Cornejo et al. 2021 [98]	C.S.	99 (in total)	All: 10.0 (1.1)	N.S.	BMI: All: 26.7 (3.7)	39	fMRI	Resting state		ROI/ Whole-brain analysis	Children with OW/OB: speed-agility ↑ , rs-FC of the **posterior hippocampus** and **precentral gyrus** ↓ , **ACC** ↓ ; cardiorespiratory fitness ↑ , rs-FC of **the anterior hippocampus** ↑ , **superior frontal gyrus** ↑
Cadenas-Sanchez et al. 2023 [99]	C.S.	110 (in total)	All: 10.0 (1.1)	N.S.	BMI: All: 26.7 (3.6)	36	Structural MRI			ROI analysis	Children with OW/OB: Physical activity ↑ , radial distance in the **putamen**, **thalamus**, **pallidum** ↑ ; cardiorespiratory fitness ↑ , radial distance in the **amygdala** ↑ ; speed-agility ↑ , radial distance in the **NAcc** ↑
Logan et al. 2022 [100]	C.S.	121 (in total)	All: 9.3 (1.1)	1.44 ± 0.5	BMI: All: 19.0 (4.2)	56	fMRI	Resting state		ROI analysis	Cardiorespiratory fitness ↑ FC between regions in the **ventral attention** and **frontoparietal networks**
Rodriguez-Ayllon et al. 2020 [101]	C.S.	103 (in total)	All: 10.02 (1.15)	N.S.	BMI: All: 26.72 (3.62)	41	DTI			Whole-brain analysis	Children with OW/OB: physical activity ↑ , **global FA** ↑
Brooks et al. 2021 [102]	C.S.	5955 (in total)	9-10	N.S.	BMI: All: 17.37 (4.28)	51	fMRI	Resting state		Whole-brain analysis	Physical activity ↑ , node clustering ↑ , reflecting the efficiency of within-network, of the **DMN**, **ECN** and **SN**

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Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI %/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Alves et al. 2021 [103]	C.S.	100 (in total)	All: 8.51 (1.00)	1-3	BMIz: All: 0.75 (1.09)	59	DTI			Whole-brain analysis	Children exposed to maternal OW/OB: vigorous physical activity ↑, **global FA** ↑ and intelligence ↑
Rodriguez-Ayllon et al. 2020 [104]	C.S.	104 (in total)	All: 10.04 (1.15)	N.S.	BMI: All: 26.68 (3.63)	41	DTI			Whole-brain analysis	Physical fitness showed no association with global FA, but muscular fitness ↑, FA in the **lateral frontal lobe** ↑
Adelantado-Renau et al. 2023 [105]	C.S.	100 (in total)	All: 10.0 (1.2)	N.S.	BMI: All: 26.6 (3.5)	41	Structural MRI			ROI analysis	Children with OW/OB: No association between neurotrophic factors and **hippocampal** volume
Ortega et al. 2022 [107]	L.S.	109 (in total)	All: 10.0 (1.1)		BMI: All: 26.8 (3.6)	41	Structural MRI			ROI analysis	Children with OW/OB: No significant effect of exercise on the volume of the **hippocampus**, the shape of **DS**, **VS**, **amygdala**
Krafft et al. 2014 [108]	C.S.	18 (Exercise group: 10; Control group: 8)	Exercise group: 9.9 (0.6); Control group: 9.4 (0.8)	N.S.	BMI: Exercise group: 25.6 (3.7); Control group: 27.2 (10.4)	50	DTI			ROI analysis	Exercise attendance ↑, FA ↑, RD ↓ of **superior longitudinal fasciculus**

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Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI %/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Schaeffer et al. 2014 [109]	C.S.	18 (Exercise group: 10; Control group: 8)	Exercise group: 9.9 (0.6); Control group: 9.4 (0.8)	N.S.	BMI: Exercise group: 25.6 (3.7); Control group: 27.2 (10.4)	50	DTI			ROI analysis	Children with OW/OB: FA ↑, RD ↓ in the **uncinate fasciculus**
Krafft et al. 2014 [110]	C.S.	43 (Exercise group: 24; Control group: 19)	Exercise group: 9.7 (0.8); Control group: 9.9 (0.9)	N.S.	BMIz: Exercise group: 1.91 (0.42); Control group: 1.93 (0.57)	65	fMRI	Antisaccade + flanker task	N.S.	Whole-brain analysis	Exercise group: activation in the **precentral gyrus** ↓ and **posterior parietal cortex** ↓, activation in the **ACC** ↑, **superior frontal gyrus** ↑
Krafft et al. 2014 [111]	C.S.	22 (Exercise group: 13; Control group: 9)	Exercise group: 9.5 (0.6); Control group: 9.6 (0.9)	N.S.	BMI ≥ 85th percentile	68	fMRI	Resting state		ROI analysis	Exercise group: between-networks synchrony in **DMN** ↓, **ECN** ↓, **motor network** ↓
Davis et al. 2011 [112]	C.S.	18 (Exercise group: 11; Control group: 9)	All: 9.6 (1.0)	N.S.	BMI: All: 25.3 (6.0)	40	fMRI	Antisaccade task	N.S.	ROI analysis	Children with OW/OB: exercise ↑, activation in the **PFC** ↑, **posterior parietal cortex** ↓

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Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI %/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Leidy et al. 2011 [114]	L.S.	10 (in total)	All: 15 (1)	N.S.	BMI%: All: 93.1 (1.4)	100	fMRI	Visual food cue	Eat 3 h prior fMRI	Whole-brain analysis	Adolescents with OW: brain responses to food (vs. non-food) cues in the **amygdala** ↓, **ACC** ↓, **hippocampus** ↓, **parahippocampus** ↓, after breakfast consumption
Hinton et al. 2018 [115]	L.S.	19 (in total)	11-18	N.S.	BMI ≥ 95th percentile	63	fMRI	Visual food cue	N.S.	ROI analysis	Adolescents with OB: after a meal weighing device training, reactivation to food cue in **striatum** ↓, **temporal occipital fusiform cortex** ↓ at 60/90 min following glucose consumption
Augustijn et al. 2019 [116]	L.S.	43 (24 HW, 19 OB)	pre HW: 9.6 (1.2); OB: 9.4 (1.0); post HW: 10.0 (1.2); OB: 9.8 (1.0)	1-3	BMI: pre HW: 16.90 (1.17); OB: 31.50 (4.43); post HW: 16.91 (1.21); OB: 25.71 (3.73)	37	Structural MRI			Whole-brain analysis	OB children with 5-months intervention: the grey matter volume of **cerebellar** ↑ and **total brain** ↑
Mata et al. 2016 [117]	L.S.	16 (in total)	All: pre: 13.94 (1.65)	N.S.	BMI: pre: 27.95 (4.29); post: 26.46 (4.37)	75	fMRI	Risky-Gains Task	N.S.	ROI analysis	BMI and fat mass reduction ↑, signal change in **anterior insula** ↑ between pre- and post-intervention during risky decision-making

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Author	Study design	Study sample	Age (M, SD)/age range	Tanner stage (M)	Weight status: BMIz/ BMI/ BMI %/ BMI cole score (M, SD)	Gender (% female)	Imaging modality	Paradigm	Fasting before fMRI	Analysis methods	Main outcomes
Kinder et al. 2014 [118]	L.S.	14 (in total)	pre: 13.15 (2.51); post: 14.48 (2.44)	N.S.	BMI: pre: 28.89 (3.80); post: 28.53 (4.28)	86	fMRI	Visual food cue	N.S.	ROI analysis	Therapeutic responder group: activation in the **putamen** ↑ , **insula** ↑ , **motor pattern storage regions (i.e., the inferior frontal gyrus, the ventral premotor cortex)** ↑ to foods, pleasure, and sports images, respectively
Schur et al. 2020 [119]	L.S.	37 (in total)	All: 10.5 (0.9)	N.S.	BMI: pre: 29.5 (7.0); post: 28.1 (7.6)	38	fMRI	Visual food cue	N.S.	ROI/ whole-brain analysis	Reductions of averaged activation after a meal in the **amygdala** ↑ , **DS** ↑ , **VS** ↑ , **insula** ↑ , **medial OFC** ↑ , **substantia nigra/ventral tegmental area** ↑ to high-calorie food, BMI declines ↑ after a 6-month intervention
Martín-Pérez et al. 2020 [120]	L.S.	70 (36 HW, 34 OW/OB)	HW: 16.50 (1.40); OW/OB: 16.44 (1.66)	N.S.	BMI%: HW: 50.33 (19.31); OW/OB: 93.74 (4.27)	54	fMRI	Resting state		ROI analysis	Adolescents with OW/OB: rs-FC **between the left central amygdala nuclei and midbrain** at baseline ↑ , weight loss ↓ after 3-months of dietary/exercise intervention

Note. " ↑ " , increased; " ↓ ", decreased; ACC = anterior cingulate cortex; BMI = body mass index; BMIz = BMI z-score; C.S.= cross-sectional study; DTI = diffusion tensor imaging; DS = dorsal striatum; dlPFC = dorsolateral prefrontal cortex; DMN = default mode network; ECN = executive control network; fMRI = functional MRI; FC = functional connectivity; FA = fractional anisotropy; HW = healthy weight; L.S.= longitudinal study; M = mean; MD = mean diffusivity; MRI = magnetic resonance imaging; N.S. = not specified; NAcc = nucleus accumbens; OW = overweight; OB = obesity; OFC = orbital frontal cortex; PFC = prefrontal cortex; ROI: region of interest; RD = radial diffusivity; rs-fMRI = resting state fMRI; SD = standard deviation; SN = salience network; VS = ventral striatum.

2.2 Exposure to Gestational Diabetes Mellitus in Utero Impacts Hippocampal Functional Connectivity in Response to Food Cues in Children (Chapter 2)

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Chapter 2 focuses on the long-term effects of intrauterine hyperinsulinemia exposure on hippocampal development. Hippocampal functional connectivity during a food cue task was compared between children aged 7–11 years who were exposed to GDM and those not exposed, adjusting for current adiposity measures (i.e., waist-to-hip ratio). The study design is illustrated in **Figure 2.**
Children exposed to GDM exhibited increased hippocampal functional connectivity with regions involved in reward processing compared to non-exposed children. Additionally, we explored the associations between children's BMI and hippocampal functional connectivity in both GDM-exposed and Non-exposed groups to understand the potential influence of obesity. Notably, a negative correlation between hippocampal connectivity and the somatosensory cortex was found exclusively in children with GDM exposure.

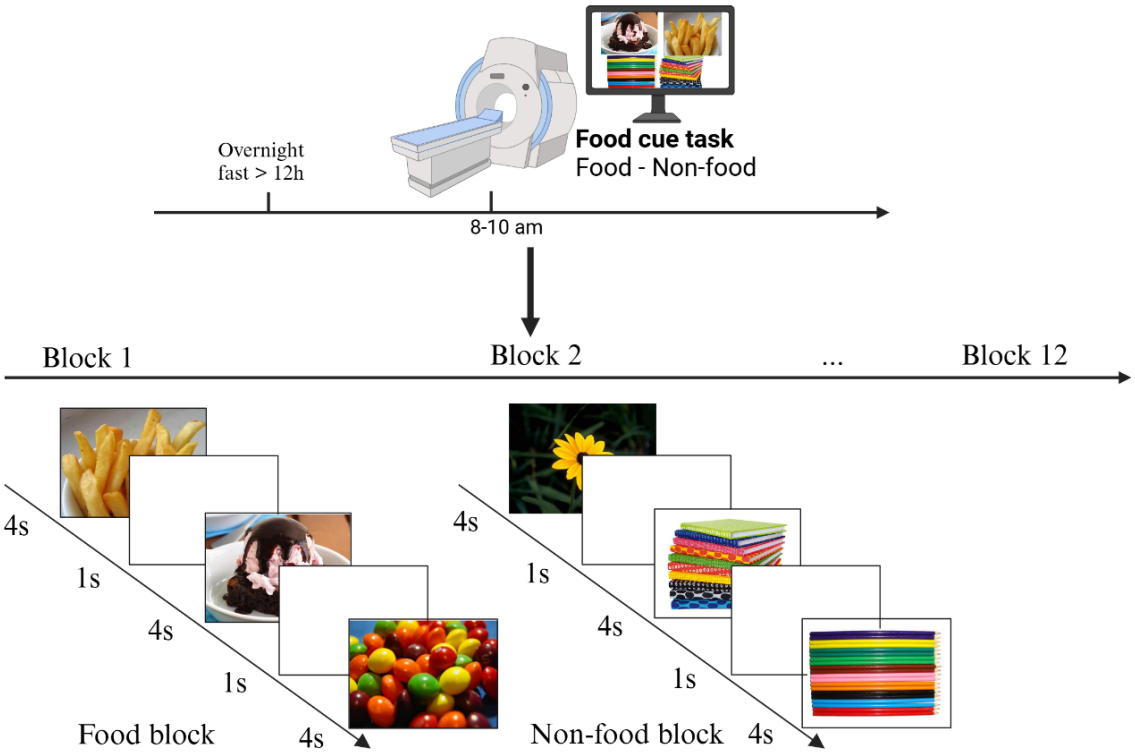

Figure 2

Study design of **Chapter 2**. Figure was created with BioRender.com.

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Pediatrics

Exposure to gestational diabetes mellitus in utero impacts hippocampal functional connectivity in response to food cues in children

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OBJECTIVES: Intrauterine exposure to gestational diabetes mellitus (GDM) increases the risk of obesity in the offspring, but little is known about the underlying neural mechanisms. The hippocampus is crucial for food intake regulation and is vulnerable to the effects of obesity. The purpose of the study was to investigate whether GDM exposure affects hippocampal functional connectivity during exposure to food cues using functional magnetic resonance imaging (fMRI).
METHODS: Participants were 90 children age 7–11 years (53 females) who underwent an fMRI-based visual food cue task in the fasted state. Hippocampal functional connectivity (FC) was examined using generalized psychophysiological interaction in response to food versus non-food cues. Hippocampal FC was compared between children with and without GDM exposure, while controlling for possible confounding effects of age, sex and waist-to-hip ratio. In addition, the influence of childhood and maternal obesity were investigated using multiple regression models.
RESULTS: While viewing high caloric food cues compared to non-food cure, children with GDM exposure exhibited higher hippocampal FC to the insula and striatum (i.e., putamen, pallidum and nucleus accumbens) compared to unexposed children. With increasing BMI, children with GDM exposure had lower hippocampal FC to the somatosensory cortex (i.e., postcentral gyrus).
CONCLUSIONS: Intrauterine exposure to GDM was associated with higher food-cue induced hippocampal FC especially to reward processing regions. Future studies with longitudinal measurements are needed to clarify whether altered hippocampal FC may raise the risk of the development of metabolic diseases later in life.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is traditionally defined as glucose intolerance with first-time diagnosis during pregnancy [1]. It develops in approximately 10% of pregnancies, making it one of the prevalent complications during gestation [2]. Intrauterine exposure to GDM increases the risk of developing obesity in offspring [2]. It is not yet clear which factors might drive these conditions later in life, but early neurodevelopmental processes appear sensitive to intrauterine hyperglycemia, hyperinsulinemia and neuroinflammation caused by maternal overnutrition, including hyperglycemia [3, 4]. Furthermore, intrauterine exposure to GDM may lead to increased food intake, which is regulated by multiple brain regions, as the hypothalamus, striatum, insula, hippocampus etc. [5, 6]. Significantly, functional imaging data demonstrated that food cue reactivity in these brain regions can predict weight gain including in children [7, 8].

Children exposed to GDM display higher food cue reactivity in the orbitofrontal cortex [9], fail to inhibit hypothalamic activity after glucose ingestion [10] and exhibit hypothalamic inflammation [11]. Moreover, data from animals and humans suggests the development of the hippocampus is sensitive to adverse in utero environmental exposures (e.g., GDM) [4, 12–15]. In animals, intrauterine exposure to diabetes caused decreased neuronal density and reduced synaptic integrity in the hippocampus [4, 12, 13]. GDM exposure in utero and maternal obesity also

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associated with reduced thickness and volume in the hippocampus in children [14, 15].

The hippocampus is known for its major role in learning and memory and is believed to influence food intake by integrating learned experiences with interoceptive signals (for review, see [16]). Animal models and behavioral studies in humans suggest that even a brief exposure to a diet rich in dietary fat and sugar can impair hippocampal-dependent learning and memory [17, 18]. Behavioral data in healthy humans showed that influencing meal memory can reduce or enhance later food intake [19, 20]. Furthermore, amnesic patients fail to interpret interoceptive signals related to hunger and satiety [21]. Using fMRI, the hippocampus has been shown to be responsive to the ingestion of sugar, visual food cues, and postprandial hormones in healthy adults [16, 22, 23]. Hence, hippocampal dysfunctions may impair the ability to retrieve memories of meals, detect interoceptive signals, which may lead to overeating (for reviews, see [24]).

However, there is currently no available research on the hippocampus functional network in response to visual food cues in children with GDM exposure, who exhibit higher risk of developing obesity [2]. Thus, the current study investigates the relation between GDM exposure and functional connectivity (FC) of the hippocampus in children.

We examined task-based FC of the bilateral hippocampus in children with and without GDM exposure using generalized psychophysiological interaction (gPPI) in response to visual food cues (food minus non-food) in the BrainChild Cohort [9, 25]. Prior studies [26–32] indicate higher food-cue-induced neural reactivity of reward regions and alterations in hippocampal FC in both children and adults with obesity. Hence, we hypothesized that hippocampal FC is higher to reward-related regions during food cue presentation in children with GDM exposure when compared to children without exposure. In addition, we explored the relationship between adiposity measures of children and mothers and hippocampal FC. Given prior evidence suggesting that GDM has distinct effects on the left and right hippocampus in children [14], we conducted separate exploratory analyses on the FC of the left and right hippocampus.

METHODS

Participants

Participants included 112 children from the larger BrainChild study assessing the impact of exposure to GDM in utero on neural and endocrine systems underlying risk for obesity and diabetes [10]. The BrainChild study included typically developing children aged 7–11 years recruited from Kaiser Permanente Southern California (KPSC) [9, 25]. Inclusion criteria included KPSC's electronic medical records, which documented maternal GDM or normal glucose tolerance during pregnancy, uncomplicated singleton birth, and children with no history of medical/psychiatric disorders or taking medicines affecting metabolism. Twenty-two participants were excluded due to excessive movement, image artifacts, or the presence of brain lesions. The final analyses included a total of 90 participants. Based on the sample size of $N = 90$ and the detected effect size of 0.8 (primary analysis: GDM versus Non-GDM), we achieved a statistical power of 0.96 at an alpha level of 0.05.

Ethics approval and consent to participate

The institutional review board at both KPSC (# 10282) and University of Southern California (USC) (# HS-14-00034) approved this study. This study was in accordance with the Declaration of Helsinki. Parents and children were provided with written informed consent and informed child assent prior to the study.

Maternal GDM exposure

GDM during pregnancy was determined based on one of the following laboratory plasma glucose values during pregnancy: (1) plasma glucose values ≥ 200 mg/dL from a 50 g 1-hr glucose challenge test, (2) at least two plasma glucose values meeting or exceeding the following values on either the 75 g 2-hrs or 100 g 3-hrs oral glucose tolerance test: fasting, 95 mg/dL; 1 h, 180 mg/dL; 2 h, 155 mg/dL; and 3 h, 140 mg/dL [33].

Study procedures

The data for this study were collected over two visits conducted after a 12-h overnight fast. The first visit consisted of metabolic phenotyping, including assessments of anthropometric measures. The second visit was a neuroimaging visit, including functional magnetic resonance imaging (fMRI) measurement during a food cue task after the overnight fast.

First visit: anthropometric measurement

During the first visit, anthropometric data, including height, weight, waist and hip circumferences of both the mother and child, tanner stage of child were collected at the Clinical Research Unit of the USC Diabetes and Obesity Research Institute as previously reported [10]. Specific to children, BMI *z*-scores (BMI-z) were calculated using the Center for Disease Control (CDC) guidelines [34].

Second visit: MRI measurement

After the overnight fast, fMRI measurements of the children were performed at the USC Dana and David Dornsife Neuroimaging Center. Children first underwent training on a mock scanner, after which they were imaged in a 3 T MRI scanner. All children were scanned between 8 and 10 am following 12-h of overnight fasting. They completed a visual food cue task in the scanner (For more details, see [25]). Briefly, children were presented high-calorie food (e.g., ice cream) and non-food (e.g., pencils) pictures and instructed to watch the pictures attentively. The stimuli were selected based on pilot studies of children's ratings of familiarity and appeal of the food and non-food items. And, the food and non-food tems were also selected to include similar characteristics such as contrast, salience, color, shape and complexity. A total of 12 blocks of stimuli were included, comprising an equal distribution of 50% food images and 50% non-food images. Each block included three images and each image was displayed for 4 s with 1 s consistent inter-stimulus interval between pictures. The sequence of the blocks was randomized. The food cue task lasted 196 s in total. The task was designed to be particularly efficient for differential effects (food versus non-food) with a short stimulus onset asynchrony and not for common task effects or task effects versus implicit baseline.

Image acquisition and preprocessing

The imaging was conducted on a Siemens MAGNETOM Prismafit 3 T MRI scanner with a 20-channel head coil. Functional images were obtained using a 2D single-shot gradient echo planar imaging sequence with the following parameters: repetition time (TR) = 2000 ms; echo time = 25 ms; flip angle = 85°; voxel resolution $3.4 \times 3.4 \times 4\ mm^3$; 32 axial slices. A high-resolution structural image was also acquired at $1 \times 1 \times 1\ mm^3$ resolution. For more details, see publication [25].

The preprocessing of the fMRI data was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Slice timing and realignment were performed for each fMRI time series. Movement criteria was movement > 2° or 2 mm in any direction, or mean framewise displacement of more than 0.3 mm. The resulting mean functional image and the structural image was coregistered. Unified segmentation was performed to the anatomical image and normalization parameters were estimated. Then, these parameters were applied to the functional images and normalized into Montreal Neurological Institute (MNI) space, using the same method applied in our previous paper by Luo et al. [25] and in other studies [35, 36] with children within the same age range. The data were then smoothed with an 8 mm field-width half-maximum (FWHM) Gaussian kernel. Physiological noise signals in the white matter and cerebrospinal fluid were extracted using Principal Component Analysis (PCA) using the PhysIO toolbox [37].

Region of interest (ROI) definition

To specifically investigate the effect of GDM on the hippocampus FC, we used an anatomical ROI-based approach. Left, right and bilateral ROIs of the hippocampus were created using the AAL atlas 3 (AAL3, https://www.oxcns.org) (Fig. 1).

Generalized psychophysiological interaction (first level analysis)

For each participant, the brain response to high-calorie food and non-food images was convolved with a canonical hemodynamic response function, and then added to the General Linear Model (GLM). The six motion parameters, and three components each of the white matter and cerebrospinal fluid signals extracted by PCA were also included in the

GLM as confounds. High-pass filtering was applied using bandwidth = 0.0078 (1/128) Hz.

Task-based FC between anatomical seed region of the hippocampus (i.e., bilateral hippocampus) and all other brain voxels was assessed using a generalized psychophysiological interaction (gPPI) approach (https://www.nitrc.org/projects/gppi version 13.1). In an exploratory analysis, FC was assessed for the right and left hippocampus separately in the same way.

First, the time series from the seed region were extracted. Second, the PPI interaction terms were generated for food and non-food stimuli according to the time series. Finally, FC of the seed region was computed for food and non-food stimuli for each participant.

Statistical analyses

Hippocampal functional connectivity in response to food minus non-food cues. To evaluate intrauterine exposure to GDM on food-cue induced hippocampal FC, the gPPI contrast maps of *food minus non-food* were entered into a second-level two-sample t-test model with the GDM exposure (GDM vs. Non-GDM) as grouping factor. Age and sex were included in the model as covariates due to their potential effects on hippocampal structure and function [14, 38]. Waist-to-hip ratio (WHR) rather than BMI has been reported to be positively correlated with hippocampus activity in response to food cues [39] and we recently reported higher WHR in children with GDM exposure [9]. Therefore, WHR was adjusted for the possible impact of adiposity.

The statistical parametric maps were thresholded using an uncorrected threshold of $p < 0.001$ and a cluster-level family-wise error (FWE) corrected threshold of $p < 0.05$. In addition, small volume correction (SVC) was performed for the insula and striatum (caudate, putamen, nucleus accumbens, pallidum), based on their activation in response to food reward processing and influenced by obesity in children and adolescents [40, 41]. The striatal mask and the insular mask were generated based on AAL3 (https://www.oxcns.org) and the wfu pick atlas (https://www.nitrc.org/projects/wfu_pickatlas/). Multiple comparison was implemented for two masks using corrected threshold $p < 0.025$.

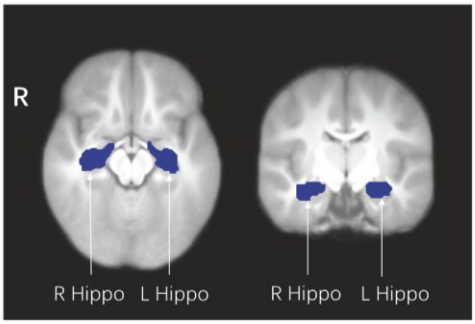

Fig. 1 Masks of the hippocampus derived from the AAL atlas 3, overlaid on the average normalized T1 weighted image of the children. Hippo, Hippocampus; L, left; R, right.

Correlation between task-based hippocampal functional connectivity and obesity measures of children and mothers. To explore the effect of children's obesity and maternal adiposity on bilateral hippocampal FC in children, a second-level multiple regression model was created using SPM 12 at the whole-brain level. This analysis was performed separately for children with and without GDM exposure. These models included the gPPI *food minus non-food* contrast as intercept, with WHR, BMI *z*-score, maternal current BMI or maternal prepregnancy BMI as the regressors of interest, adjusted for age and sex. An uncorrected threshold of $p < 0.001$ and a cluster-level FWE corrected threshold of $p < 0.05$ were used. The correlations were assessed for the right and left hippocampus separately in the same way.

RESULTS

Demographics

The demographics of the 90 participants included in this study are shown in Table 1 (ages 7–11 years, 53 females, 50 GDM exposed), and 89% of children were in Tanner Stage 1. There were no significant differences in children's age, sex, BMI *z*-score, or maternal current BMI or maternal prepregnancy BMI among GDM exposed vs. unexposed groups ($p > 0.05$, Table 1). There was a trend towards a higher WHR for children exposed to GDM than unexposed (t [88] = 1.97, $p = 0.052$, Table 1).

Hippocampal functional connectivity in response to food minus non-food cues

We observed higher FC in children with GDM exposure compared to children without GDM exposure between the bilateral hippocampus and the left insula ($p_{FWE} = 0.037$) and left putamen, which extended to the left pallidum ($p_{FWE} = 0.019$, SVC) (Table 2, Fig. 2).

In an exploratory analysis, FC was assessed for the right and left hippocampus separately. In children with GDM exposure compared to children without exposure, we observed higher FC between the left hippocampus and the right putamen ($p_{FWE} = 0.007$), left putamen ($p_{FWE} = 0.017$, SVC), right insula ($p_{FWE} = 0.017$), left insula ($p_{FWE} = 0.011$, SVC), and left nucleus accumbens (NAcc, $p_{FWE} = 0.013$, SVC) (Table 2, Fig. 2). The cluster of the right putamen extended to the right insula. The cluster of the left putamen extended to the left pallidum. No group differences were found for the right hippocampus.

Table 1. Demographics*.

	Overall 90	Non-GDM *N* = 40	GDM *N* = 50	t/Z	*p*
Children					
Age (years)	8.23 (7.82, 9.08)	8.61 (7.75, 9.67)	8.14 (7.85, 8.63)	1.406	0.30
Sex					0.13
Female	53 (58.9%)	20 (50%)	33 (66%)		
Male	37 (41.1%)	20 (50%)	17 (34%)		
BMI *z*-score	0.66 (0.03, 1.67)	0.59 (-0.06, 1.68)	0.76 (0.23, 1.69)	0.285	0.43
WHR	0.87 ± 0.06	0.86 ± 0.06	0.88 ± 0.06	1.969	0.052
Mother					
Current BMI (kg/m^2)	30.27 (26.52, 35.43)	29.58 (25.60, 35.06)	30.71 (26.90, 36.11)	4.921	0.64
Prepregnancy BMI (kg/m^2)	29.07 (25.23, 33.22)	29.07 (24.61, 32.98)	28.98 (25.37, 33.51)	2.186	0.61

BMI body mass index, *GDM* gestational diabetes mellitus, *WHR* waist-to-hip ratio, *t* statistic for two-sided independent-samples *t*-test, *Z* statistic for Mann–Whitney test for data with skewed distribution.
*For continuous variables, normally distributed data (WHR) were described as mean ± standard deviation (SD); data from skewed distribution were described by the median (Q1, Q3); Categorical variable was described as *N* (%), *p* value was calculated using Chi-square test.

Table 2. Hippocampus task-based functional connectivity in response to food versus non-food cues adjusted for age, sex and WHR.

	Brain region	Hemi	MNI coordinates			Peak t	Cluster size	p_{FWE}
			x	y	z			
Seed region	GDM > Non-GDM							
Bilateral Hippo								
	Insula	L	−42	14	−7	4.31	59	0.037
	Pallidum/ Putamen	L	−15	2	2	4.29	23	0.019^{SVC}
Hippo L								
	Putamen	R	36	−1	−4	4.87	91	0.007
	Insula	R	39	−1	−4	4.20	74	0.017
	NAcc	L	−15	5	−13	4.41	5	0.013^{SVC}
	Putamen/Pallidum	L	−18	5	5	4.32	30	0.017^{SVC}
	Insula	L	−42	8	−4	4.42	20	0.011^{SVC}
Hippo R								
	No differential activation							
	Non-GDM > GDM							
	No differential activation							

FWE family wise error, *GDM* gestational diabetes mellitus, *Hemi* hemisphere, *NAcc* nucleus accumbens, *WHR* waist-to-hip ratio, *L* left, *R* right, *p* value FWE corrected using whole-brain cluster correction, *SVC* p_{FWE} small volume corrected for ROIs.

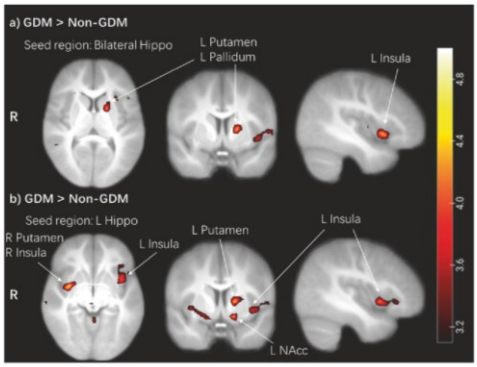

Fig. 2 Hippocampal functional connectivity during the food-cue task. a Children with GDM exposure showed higher FC between bilateral hippocampus and left insula, left putamen/pallidum. **b** Children with GDM exposure showed higher FC between left hippocampus and the bilateral putamen, insula, and left NAcc. The cluster of the right putamen extended to the right insula. The cluster of left putamen extended to the left pallidum. Color map corresponds to T values ($p < 0.001$ uncorrected for display) overlaid on the normalized average T1 weighted image of the children. Hippo hippocampus, FC functional connectivity, GDM gestational diabetes mellitus, NAcc nucleus accumbens, L left, R right.

Association between task-based hippocampal functional connectivity and obesity measures of children and mothers

No significant correlation was observed between the FC of the bilateral hippocampus and WHR, BMI *z*-score, maternal current or maternal prepregnancy BMI in both the GDM and Non-GDM groups (all $p_{FWE\text{-}corrected} > 0.05$).

Further analysis of the FC of the left or right hippocampus separately revealed significant correlations. In the GDM group, there was a negative correlation between BMI *z*-score and the FC of the left hippocampus and the right postcentral gyrus (peak-voxel (MNI) x: 57, y: −34, z: 26); $r = -0.607$; $p_{FWE\text{-}corrected} < 0.001$) (Fig. 3). In the Non-GDM group, a positive correlation was found between the maternal current BMI and the FC of the left hippocampus to the right superior frontal gyrus (peak-voxel (MNI) x: 18, y: 59, z: −1); $r = 0.574$; $p_{FWE\text{-}corrected} = 0.001$).

DISCUSSION

The current study investigated the relationship between intrauterine GDM exposure and food cue induced hippocampal functional connectivity in children aged 7–11 years in the fasted state. Consistent with our hypothesis, children with GDM exposure compared to unexposed showed higher hippocampal FC to reward processing regions (i.e., putamen, pallidum, NAcc and insular cortex) and lower hippocampal FC to the somatosensory cortex with increasing BMI.

We observed higher functional coupling between hippocampus and striatal regions and insula in children with intrauterine GDM exposure compared to children without exposure, primarily driven by the left hippocampus. A previous structural MRI report found reduced left hippocampal thickness in children with GDM exposure compared to unexposed children [14]. Therefore, GDM may affect both the structure and function of the hippocampus.

Hippocampal neurons interact with other neurons in the mesolimbic system receiving dopamine projections to communicate rewarding properties of environmental stimuli [16, 42]. As potent rewards, palatable foods can trigger associations with reward and motivational behaviors that potentially could lead to overeating and, eventually, weight gain [42]. These food cues tend to evoke heightened memories and mental simulations of consumption in children [43]. Moreover, a meta-analysis indicated that the hippocampus-striatum connection may play a role in craving and the formation of habits associated with obesity [44]. Concomitantly, higher activation in the striatum and insula in response to food images were observed in children and adolescents with obesity compared to their healthy-weight peers [40, 41, 45]. In the resting state, higher striatal and insular network FC was also linked to eating in the absence of hunger, food craving, disinhibited eating, weight gain and obesity in both children and adults [46–49]. In the current study, no significant influence of WHR or BMI was identified on these hippocampal connections in children. However, BMI negatively correlated with the left hippocampus to the somatosensory cortex FC in children exposed to GDM, aligning with resting-state studies in children with obesity [50]. The oral somatosensory cortex is known to sense fat and food texture [51] and children and adolescents with

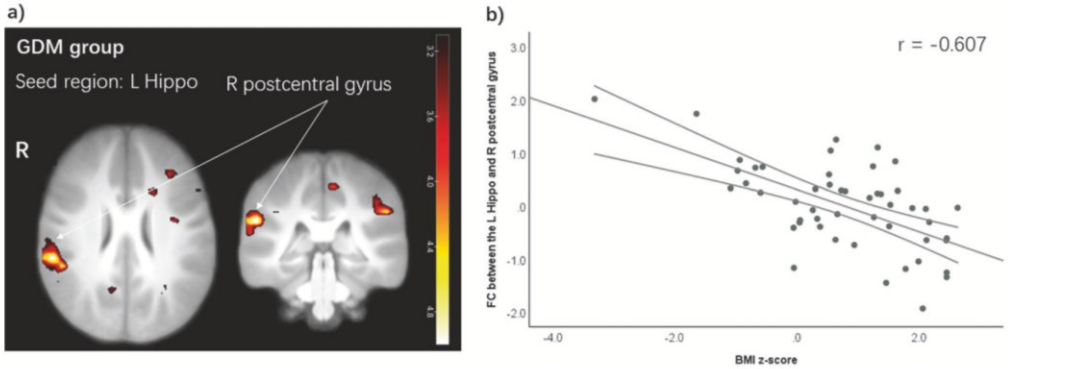

Fig. 3 Hippocampal functional connectivity in relation to BMI *z*-score in the GDM group. a Children with GDM exposure showed lower FC between the left hippocampus and the right postcentral gyrus with higher BMI *z*-score. Color map corresponds to *T* values (Multiple regression analysis with BMI *z*-score; $p < 0.001$ uncorrected for display) overlaid on the normalized average T1 weighted image of the children. **b** Negative correlation between BMI *z*-score and the extracted cluster of the FC of the left hippocampus and the right postcentral gyrus in the GDM group. Error bars indicate 95% confidence interval. Hippo hippocampus, FC functional connectivity, GDM gestational diabetes mellitus, L left, R right.

obesity show greater activation in the somatosensory cortex to food [8, 52]. The higher preference for high-fat foods in children is a predictor of future weight gain [53]. Nonetheless, it is yet unknown whether altered hippocampal to somatosensory connectivity patterns in children with GDM exposure predict the development of obesity later in life.

Our study points to a distinct effect of intrauterine GDM exposure on the hippocampal network primarily to reward processing regions, rather than obesity itself at this young age. These results align with animal studies [4, 12, 13] and provide evidence to support the hypothesis that prenatal exposure to diabetes might result in changes in brain pathways. These changes, in turn, may contribute to the increased risk of weight gain and obesity in affected children at a later age. Interestingly, previous studies suggest that hyperactivity in the brain's reward system might be a susceptibility factor for weight gain [8, 54]. Similarly, our previous study showed that children exposed to GDM had higher daily energy intake [9]. Moreover, parental obesity has been related to greater striatum and insula activation in response to food rewards and higher ad libitum intake even in adolescents of healthy-weight [8, 55]. In the current study, we also found higher food-cue induced hippocampal FC to the frontal cortex in children of mothers with higher BMI. Although this connection in relation to maternal obesity has not yet been fully investigated, higher FC between temporal and frontal regions has been reported in adolescent obesity [56]. Future studies with longitudinal measurements are necessary to evaluate whether hippocampal changes in FC result in weight gain and raise the risk of developing obesity later in life.

Our study includes some limitations. Given the limited size of our sample, each subgroup, based on GDM exposure, included a relatively small number of subjects. In addition, food intake and behavioral assessments were not assessed, and future studies are necessary to provide a more detailed understanding how the observed functional alterations in the hippocampus are related to cognitive and metabolic processes. Moreover, longitudinal data are needed to examine the association between functional alterations in the hippocampus and future weight gain in children.

CONCLUSION

Our study suggests that intrauterine exposure to GDM alters hippocampal food cue processing network in children. During palatable food picture presentation, children with GDM exposure exhibited higher hippocampal connectivity specifically to reward processing regions and lower hippocampal connectivity, with increasing BMI, to the somatosensory cortex. These alterations may be associated with a potential risk for future weight gain. Longitudinal research is required to determine if altered hippocampal functional connectivity during exposure to food cues leads to future weight gain and a higher likelihood of metabolic disorders, including obesity.

DATA AVAILABILITY

Data is available upon reasonable request from KAP.

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AUTHOR CONTRIBUTIONS

SXZ and SK conceptualized and conducted the analysis, drafted the manuscript; RV and LS supported the analysis and discussed the results; HP, AHX, KAP and SK provided critical review and revisions to the manuscript; AHX and KAP conceptualized the original study, have full access to all data in the study and take responsibility for the integrity of the data; SL, BCA, and TC managed and coordinated the study execution; ALB, HP, SK supervised the work. All authors discussed the results and implications, reviewed and edited the manuscript and approved its final version. KAP, AHX and SL provided funding for this study.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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CHAPTER 3

2.3 Sex Differences in Insulin Induced Hippocampus Functional Connectivity during Visual Food Cue Presentation (Chapter 3)

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Chapter 3 examines the effects of central insulin on hippocampal functional connectivity and sex differences while food cue processing in adults, as shown in **Figure 3**. Study 1 compares insulin-induced functional connectivity during the task between women and men. Study 2 investigates the correlation between insulin-mediated hippocampal connectivity during food cue processing and sex hormone levels in women with natural menstrual cycles, analyzed separately in the follicular and luteal phases.

Following intranasal insulin administration, hippocampal functional connectivity with the inhibitory control region increased independently of sex, which was associated with a stronger reduction in hunger and food cravings. Sex differences were observed in the hippocampal connectivity with the visual processing cortex, with men showing higher connectivity than women. This response was linked to a greater decrease in food desire in men. Additionally, we investigate the potential role of sex hormonal action throughout the menstrual cycle in modulating these connectivity differences in premenopausal women. In the luteal phase, hippocampal functional connectivity with visual processing regions was negatively associated with the estradiol/progesterone ratio, an effect not observed in the follicular phase.

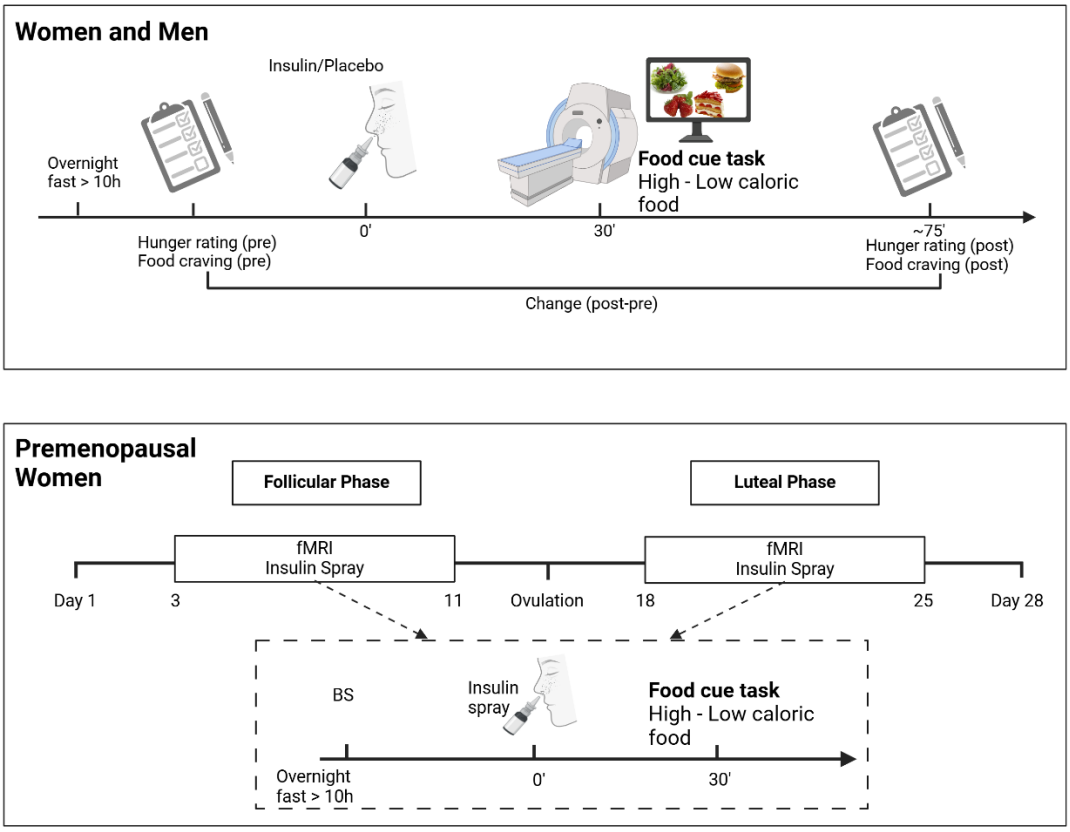

Figure 3

Study design of **Chapter 3**. Adapted from (Wagner *et al.*, 2022; Hummel *et al.*, 2023). Figure was created with BioRender.com.

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Sex Differences in Insulin-Induced Hippocampus Functional Connectivity During Visual Food-Cue Presentation

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Abstract

Context: Central insulin has been shown to regulate eating behavior and cognitive processes in a sex-specific manner. Besides memory, the hippocampus is pivotal in the control of appetite.

Objective: This work aimed to investigate how insulin interacts with the hippocampal food-cue response and evaluate the potential role of sex hormones.

Methods: Using functional magnetic resonance imaging, we evaluated task-based functional connectivity (FC) of the hippocampus during food-cue presentation in 60 participants (age: 21-69 years; 30 women) after intranasal insulin or placebo administration, in a randomized within-subject design. In an exploratory analysis, we investigated whether hippocampal FC after intranasal insulin administration is related to estradiol and progesterone levels during the follicular and luteal phase of the menstrual cycle in 13 premenopausal women (age: 20-28 years).

Results: Intranasal insulin increased hippocampal FC with the prefrontal cortex compared to placebo, regardless of sex. This correlated with stronger reduction in subjective feeling of hunger and food craving. Moreover, we observed an interaction between sex and nasal spray condition with higher hippocampal FC to the calcarine gyrus after insulin compared to placebo in men, while women showed a lower response. In premenopausal women, the centrally mediated effect of insulin on hippocampus to calcarine gyrus FC negatively correlated with the estradiol/progesterone ratio in the luteal phase.

Conclusion: Central insulin influences hippocampal FC to regions vital for inhibitory control during high-caloric food-cue presentation, implying a potential role of the hippocampal network in modulating insulin's anorexic effects. The observed sex differences between the hippocampus and visual cortex might be influenced by sex hormone action.

Key Words: hippocampus, fMRI, insulin, sex

Abbreviations: BMI, body mass index; dlPFC, dorsolateral prefrontal cortex; FC, functional connectivity; FCQ-S, Food Craving Questionnaire-State; fMRI, functional magnetic resonance imaging; FEW, family-wise error; gPPI, generalized psychophysiological interaction; PFC, prefrontal cortex; ROI, region of interest; SVC, small volume correction.

The dual function of the hippocampus in the memory and body state regulation is increasingly acknowledged (for review, see (1)). For instance, the hippocampus can inhibit food intake by detecting physiological state of satiety and hunger, also through (neuro)hormonal signals including insulin, and encode food-related memories (for reviews, see (2, 3)). In addition, central insulin action influences food intake, memory, mood, and peripheral metabolism (for reviews, see (4, 5)).

In humans, intranasal insulin administration allows the investigation of central insulin action with only minimal systemic absorption (5). Intranasal insulin influences the reactivity and functional connectivity (FC) of the mesocorticolimbic system (4), including the hippocampus, amygdala, striatum, and prefrontal cortex (PFC) (6-11). Compromised insulin action in the mesocorticolimbic system is related to greater preference for palatable food in persons with obesity-associated insulin

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resistance (7, 12, 13). Moreover, central insulin decreases the feeling of hunger in part by enhancing hippocampal FC (14, 15). It thus seems that central insulin regulates food reward behavior through the mesocorticolimbic system (4).

Behavioral studies in animals and humans have shown a sex disparity in central insulin's regulation both of food intake and memory: Males reduced food intake after acute administration of intranasal insulin in the fasted state and lost weight after 8 weeks of intranasal insulin delivery (16-18), while females reduced snacking in the postprandial state and showed improved memory on acute administration of intranasal insulin (16, 19, 20). Moreover, our previous imaging studies showed decreased central insulin action in the hippocampus with increasing age in women but not men (21) and higher centrally insulin-mediated effects on food-cue responsiveness in women in the dorsolateral prefrontal cortex (dlPFC) (15). Animal studies have suggested a link between estrogen and these sex-dependent effects of central insulin (22). In contrast, human studies have found no interaction between insulin and estrogen on food intake and several hippocampus-dependent memories (eg, visuospatial and declarative memories) after intranasal insulin administration (23-25). However, verbal recognition memory was improved, indicating an interaction between estrogen and insulin signaling on hippocampus-PFC–dependent cognitive processes (25). Additionally, in women, the response of mesolimbic regions (eg, hippocampus) to food cues and to changes in postprandial insulin levels varies across menstrual cycle phase (26-30), suggesting a potential role of sex hormone action (29).

However, it remains unclear how central insulin affects the hippocampal network and communication to other brain regions during high-caloric food-cue presentation, and whether there is a sex difference. Additionally, it also remains unknown whether sex hormones are related to a distinct pattern of hippocampal FC.

In the present study, we investigated the effect of central insulin on food-cue–induced hippocampal FC and potential sex-specific patterns, as the functional activation of specific brain regions does not exist in isolation and may indirectly influence the functioning of other regions (31). We measured central insulin action in the hippocampal FC through intranasal insulin administration. Based on previous studies (14, 32), we hypothesized that central insulin increases FC between the hippocampus and regions involved in cognitive control, such as dlPFC. Based on previous findings (16-18), we expected a higher response in men than in women. In exploratory analyses, the relationship between insulin-induced hippocampal FC and hunger rating, food-craving measures were investigated. Finally, in a separate group, we also evaluated the relationship between food-cue induced FC in the hippocampus after intranasal insulin administration and estradiol and progesterone levels in premenopausal women.

Materials and Methods

Participants

Seventy participants were enrolled in the study, as recently reported (study 1, for more details, see (15)). Ten participants were excluded due to inadequate data quality, incomplete measurements, and anatomical brain abnormalities. The final analyses included 60 participants with a wide age and weight range (age range, 21-69 years, body mass index [BMI] range, 20.87-32.38, 30 women). No statistically significant differences were observed in age, BMI, or peripheral insulin sensitivity between men and women. The characteristics of participants are provided in Supplementary Table S1 (33). Sixteen other normal-weight women with natural menstrual cycles were measured once in the follicular and luteal phase (study 2, for more details, see (30)). Additionally, sex hormone levels (ie, estradiol and progesterone) were analyzed during each visit as previously described (30). Three participants from study 2 were excluded due to incomplete measurements and extreme estradiol/progesterone ratio (>3 SDs from the mean) in the luteal phase, respectively. Thirteen premenopausal women (age range, 20-28 years; BMI range, 19.21-23.39) were included in the final analysis for study 2. Participant characteristics are shown in Supplementary Table S2 (33).

None of the participants had a medical or psychiatric history, nor were they taking any medications (apart from oral contraceptives in study 1). The ethics committee of the medical faculty of the University of Tübingen approved these studies, and they were conducted in accordance with the Declaration of Helsinki. Participants provided written informed consent prior to each study (clinical trial Nos.: study 1: NCT04372849; study 2: NCT03929419).

Study Procedures

Functional magnetic resonance imaging (fMRI) data of each participant were collected over 2 visits after an overnight fast, with a time interval of 3 to 28 days between visits (for more details, see (15)). For study 1, The fMRI measurements included an intranasal insulin and placebo visit in a randomized manner.

In a separate group, premenopausal women (study 2) underwent fMRI measurement during 2 intranasal insulin visits (no placebo visit): in the follicular phase visit and the luteal phase visit (random order). The follicular and luteal phases in each participant were determined as described previously (30).

In both studies fMRI measurements were performed 30 minutes after administering the nasal spray. Before and approximately 75 minutes after applying the spray, a hunger rating questionnaire and the Food Craving Questionnaire-State (FCQ-S) was assessed (described later).

Intranasal Insulin/Placebo Application

In study 1 participants were assigned in a randomized and blinded manner to receive a total of 160 U of insulin (Insulin Actrapid; Novo Nordisk) or a placebo vehicle during each visit. In study 2 participants received 160 U of nasal insulin at each visit (follicular and luteal phases). No placebo was administered in this group.

Magnetic Resonance Imaging Measurements

All participants underwent identical fMRI measurements at each visit in a 3T MRI scanner (Siemens MAGNETOM Prismafit). The imaging was conducted with a 20-channel head coil. Functional images were obtained using a multiband accelerated echo-planar imaging sequence with the following parameters: repetition time = 1500 ms; echo time = 34 ms; flip angle = 70°; voxel resolution $2 \times 2 \times 2$ mm^3; and 72 axial slices. A high-resolution structural image was also acquired at $1 \times 1 \times 1$ mm^3 resolution.

As previously described (15), 30 minutes after nasal spray application, participants underwent a visual food-cue task in the scanner. Briefly, participants were presented high-caloric and low-caloric food pictures in a pseudorandomized order.

An event-related design including 60 standardized food pictures (30 high-caloric [eg, pizza, cakes], 30 low-caloric [eg, salads, fruits]) was used. Each image was displayed for 2 seconds with an intertrial interval of 6 to 10 seconds. The food-cue task lasted 11 minutes including 2 sessions, each lasting 5 minutes and 30 seconds.

Hunger Rating and Food Craving Questionnaire

Subjective feeling of hunger and the FCQ-S (34) were evaluated both before (pre) and 75 minutes after (post) the application of the spray. The FCQ-S is rated on a scale from 1 to 5 (strongly disagree to strongly agree), and comprises 5 subscales (desire, reinforcement, relief, control, and hunger). Individual scores were calculated for each subscale, and the total score was derived by summing up responses to all items. Subjective feeling of hunger was evaluated using a visual analogue scale ranging from 0 to 10 (not hungry at all to very hungry). For the analysis, hunger ratings and FCQ-S scores were baseline-corrected by subtracting the premeasurement rating from the postnasal spray measurement rating. The changes in hunger ratings and FCQ-S scores were determined by subtracting the baseline-corrected ratings on the placebo day from those on the insulin day, as insulin $_{\text{post-pre}}$ minus placebo $_{\text{post-pre}}$ (study 1), and luteal phase from those on the follicular phase, as follicular $_{\text{post-pre}}$ minus luteal $_{\text{post-pre}}$ (study 2).

Region of Interest Definition

An anatomical ROI-based approach was employed to specifically examine the task-based hippocampal FC. ROI of the bilateral hippocampus was created using the AAL atlas 3 (AAL3, https://www.oxcns.org).

Image Preprocessing

The preprocessing of the fMRI data was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Slice timing and realignment were performed for each fMRI time series. The criteria for exclusion due to movement was defined as movement greater than 2° or 2 mm in any direction. The mean functional image was then coregistered to the structural image and normalized into Montreal Neurological Institute (MNI) space. Data smoothing was performed with a 6-mm field-width half-maximum (FWHM) Gaussian kernel. High-pass filtering (128 seconds) and correction for global AR (1) autocorrelation were applied.

Generalized Psychophysiological Interaction (First-Level Analysis)

The brain response to high-caloric and low-caloric food images underwent convolution with a canonical hemodynamic response function before being added into the general linear model. The 6 motion parameters were also included in the general linear model as confoundings.

A generalized psychophysiological interaction (gPPI) approach (https://www.nitrc.org/projects/gppi version) was used to assess task-based FC between the seed region (ie, bilateral hippocampus) and all other voxels in the brain.

After extracting the time series from the seed region, the PPI interaction terms for high-caloric and low-caloric stimuli were generated separately for insulin and placebo days in study 1, and for the follicular and luteal phases in study 2. Subsequently, FC of the seed region was computed for high-caloric and low-caloric stimuli for each participant in both studies. Finally, the gPPI contrast maps of high-caloric minus low-caloric food were created for both the insulin and placebo days in study 1, as well as for the follicular phase and luteal phase in study 2.

Statistical Analyses

Central insulin action on hippocampal functional connectivity during high-caloric minus low-caloric food-cue presentation

To evaluate central insulin action on hippocampal FC and sex differences, a second-level full factorial model in SPM 12 was applied. This model incorporated sex (women vs men) as a between-subject factor and intranasal spray (insulin vs placebo spray) as a within-subject factor. Age was also included in the model as a covariate. The statistical parametric maps were thresholded using an uncorrected threshold of P less than .001 and a cluster-level family-wise error (FWE)-corrected threshold of Pless than .05 on a whole-brain level. In addition, small volume correction (SVC) was performed for the dlPFC, a region within the PFC known to be insulin sensitive and important for cognitive control (4). The mask was generated based on the wfu pick atlas (https://www.nitrc.org/projects/wfu_pickatlas/).

The connectivity parameters (β values) of significant clusters and voxels were extracted using REX toolbox based on MATLAB (https://www.nitrc.org/projects/rex/) for plotting of the results and further correlation analyses using IBM SPSS Statistics (version 25; IBM Corp).

Post hoc analysis of sex × spray interaction

Independent t tests were used to compare hippocampal FC between men and women within the insulin and placebo conditions separately. Paired t tests were conducted to compare the insulin and placebo conditions within each sex group. To account for multiple comparisons, the P value was adjusted using Bonferroni correction ($0.05/4 = 0.0125$).

Association between insulin-induced hippocampal network and hunger rating and food craving

Exploratory correlation analyses (study 1) were performed between the differential (insulin – placebo) hippocampal FC and hunger rating, FCQ-S total score. For regions showing a statistically significant interaction with sex, correlations were evaluated for men and women separately. For ordinal scales, Spearman correlation analysis was employed, while Pearson correlation analysis was used for continuous scales. A significance level of P less than .05 was considered statistically significant.

Association between hippocampal functional connectivity after intranasal insulin administration with sex hormone levels in premenopausal women

In an exploratory analysis, in study 2, a multiple regression model was created for the follicular and luteal phases in SPM 12 to examine the role of sex hormone in hippocampal FC in response to central insulin on a whole-brain level. This model included the high-caloric minus low-caloric food contrast as intercept and the estradiol/progesterone ratio as the regressor. The estradiol/progesterone ratio, rather than estradiol or progesterone alone, was included as an interest of regressor, as it better captures hormonal fluctuations across the menstrual cycle and has shown better predictive power for behavior (35). An uncorrected threshold of P less than

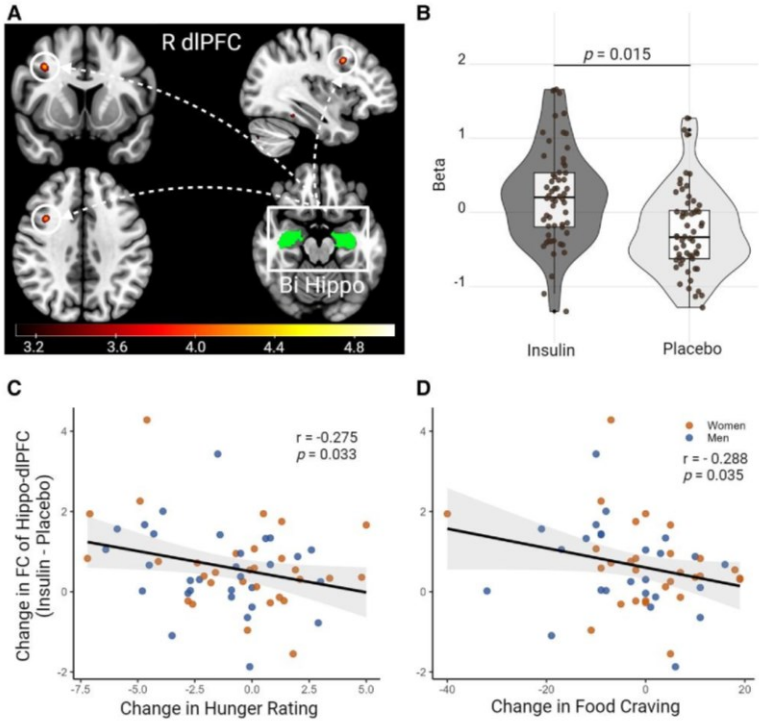

Figure 1. Central insulin action on hippocampal FC during high-caloric food-cue presentation. A, FC between the hippocampus and right dorsolateral prefrontal cortex was higher after intranasal insulin administration compared to placebo ($P_{FWE} < .05$; small volume correction). Color map corresponds to *t* values ($P < .001$ uncorrected for display) overlaid on the standardized brain image. B, The violin box plot shows the extracted β values of voxels showing higher hippocampus FC following insulin administration compared to placebo. C, Correlation plot shows a negative relationship between hippocampal FC (insulin minus placebo) and hunger ratings (insulin $_{post\text{-}pre}$ minus placebo $_{post\text{-}pre}$). D, Correlation plot shows a negative relationship between hippocampal FC (insulin minus placebo) and total food craving score (insulin $_{post\text{-}pre}$ minus placebo $_{post\text{-}pre}$). Hence, higher insulin-induced FC was related to decreased subjective hunger feeling and food craving. Figure was created using R version 4. 2. 2 and BioRender.com.

Abbreviations: Bi, bilateral; dlPFC, dorsolateral prefrontal cortex; FC, functional connectivity; Hippo, hippocampus; R, right.

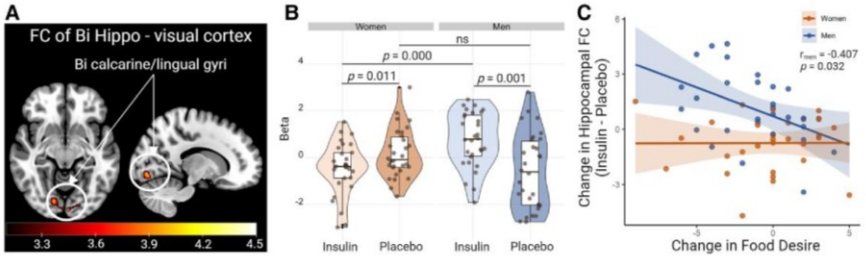

Figure 2. Sex differences in central insulin action on hippocampal FC during high-caloric food cue presentation. A, Interaction of sex × nasal spray on the FC between the hippocampus and the bilateral calcarine gyri ($P_{FWE} < .05$; whole-brain corrected). Color map corresponds to *t* values ($P < .001$ uncorrected for display) overlaid on the standardized brain image. B, Men exhibited higher FC between the hippocampus and the calcarine/lingual gyri than women after insulin administration. Furthermore, men exhibited higher FC in response to insulin compared to placebo. Women showed an opposite pattern. Bonferroni corrected threshold = 0.0125. C, In men but not women, insulin-induced changes in FC (insulin minus placebo) were negatively correlated with changes in food craving (FCQ-S subscale desire for food, insulin $_{post\text{-}pre}$ minus placebo $_{post\text{-}pre}$). Figure was created using R version 4. 2. 2 and BioRender.com.

Abbreviations: Bi, bilateral; FC, functional connectivity; Hippo, Hippocampus; ns, not significant.

have revealed the significance of the dlPFC activation in controlling appetite, promoting healthy dietary choices, and facilitating both weight loss and weight maintenance (36-39). Furthermore, regional activity in the hippocampus and dlPFC are particularly sensitive to central insulin, as seen in altered activation patterns during resting state and exposure to food cues after intranasal

Table 2. Hippocampal food-cue functional connectivity in relation to estradiol/progesterone ratio in premenopausal women after intranasal insulin administration

			MNI coordinates					
Seed region	Brain region	Hemi	x	y	z	Peak *t*	Cluster size	P_{FWE}
Negative correlation with estradiol/progesterone ratio in luteal phase								
Bilateral hippo	Calcarine gyrus	L	−24	−68	8	6.80	96	.040
	Calcarine gyrus	L	−24	−60	4	5.84		
	Superior occipital gyrus	R	26	−88	22	6.50	154	.001
	Superior occipital gyrus	R	24	−74	20	6.28		
	Calcarine gyrus	R	24	−68	14	5.25		
Positive correlation with estradiol/progesterone ratio in luteal phase								
No differential activation								
Correlations in follicular phase								
No differential activation								

P value FWE corrected using whole-brain cluster correction.
Abbreviations: FWE, family-wise error; Hemi, hemisphere; L, left; R, right.

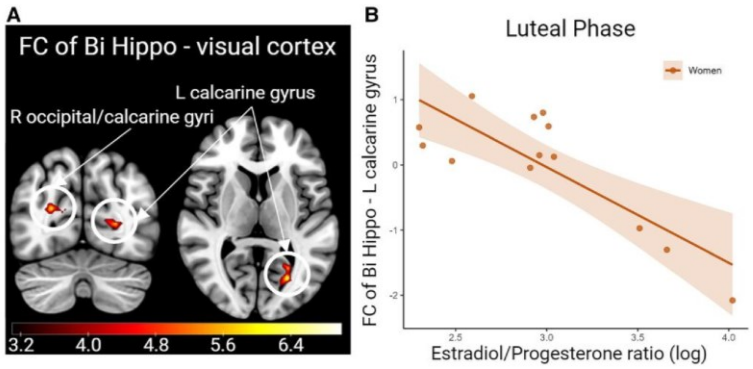

Figure 3. Central insulin action on hippocampal FC during high-caloric food cue presentation is associated with estradiol/progesterone ratio during the luteal phase of the menstrual cycle ($P_{FWE} < .05$; whole-brain corrected). A, Color map corresponds to *t* values ($P < .001$ uncorrected for display) overlaid on the standardized brain image. B, Correlation plot shows extracted FC values of the cluster of the left calcarine gyrus showing a significant negative correlation with the estradiol/progesterone ratio during the luteal phase. Figure was created using R version 4. 2. 2 and BioRender.com.

Abbreviations: Bi, bilateral; FC, functional connectivity; Hippo, hippocampus; L, left; R, right.

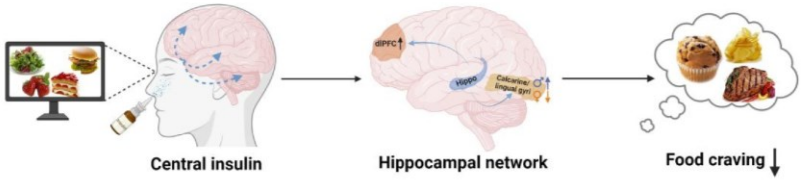

Figure 4. Central insulin might regulate food craving through the hippocampal network during visual food cue exposure. We investigated the effects of intranasal insulin on hippocampal FC during high-caloric vs low-caloric food cue presentation. Central insulin increased hippocampal FC with the right dlPFC, which was correlated with stronger reduction in subjective feeling of hunger and food craving. Sex specific findings were observed on insulin-mediated effects on hippocampal FC with the visual cortex. This hippocampal response was related to a reduction in food craving in men.

Abbreviations: FC, functional connectivity; dlPFC, dorsolateral prefrontal cortex.

insulin administration in men and women with varying body weight (9, 15, 21). This response is in part sex-specific: Women showed higher insulin action in the right dlPFC to highly desired food cues than men and lower insulin action in the hippocampus with increasing age (15, 21). However, we observed no sex differences in FC between the hippocampus and dlPFC. Additionally, participants exhibiting higher insulin-induced FC showed a stronger reduction of hunger feeling and food craving, aligning with findings that showed that hippocampus FC predicts subjective feeling of hunger (14, 40). Taken together, our

findings suggest that central insulin may diminish food consumption by enhancing the feeling of satiety and reducing food-related motivation through the hippocampus-dlPFC pathway.

In addition, central insulin action elicited a sex-dependent alteration in FC between the hippocampus and calcarine/lingual gyri (parts of the visual cortex). Specifically, men exhibited higher FC following nasal insulin application relative to women. Notably, there was no difference in peripheral insulin sensitivity between men and women, suggesting that the observed sex-specific effects of insulin on hippocampal FC are unlikely to be influenced by variations in peripheral insulin sensitivity. Our previous study has shown a sex-specific action of central insulin in the hippocampus, with women exhibiting a reduction of central insulin action with increasing age, which was not found in men (21). Similarly, functional differences in the hippocampus have been identified between sexes (for review, see (41)). For example, a stronger FC between the hippocampus and calcarine gyrus during the resting state has been observed in male patients with mild cognitive impairment (42), the prodromal stage of Alzheimer disease, which is linked to brain insulin resistance (43). This may suggest a potential sex-dependent response of this intrinsic functional connection to changes in brain insulin levels. The calcarine and lingual gyri serve as the primary visual cortex, vital for processing visual information and directing attention (44). Their involvements in food reward evaluation, high-caloric food preferences, and responses to such stimuli have been indicated in imaging studies (45-49). Moreover, the reactivity of these regions to food cues was found to be influenced by endogenous serum insulin levels after glucose ingestion (50, 51) and intranasal insulin administration (9). Additionally, we found that only men with higher FC between the hippocampus and visual-processing regions exhibited a stronger reduction in food desire after insulin administration. This effect was not observed in women. Behavioral data in humans also point toward sex differences in the anorexic effect of central insulin, showing a decrease in food intake and reduction of body fat and weight exclusively in men following acute and 8-week intranasal insulin application, respectively (16, 17). The higher FC of the hippocampus in men could potentially serve as the neural mechanism underlying central insulin action in eating behavioral effects, specifically by reducing food reactivity in men. However, whether higher hippocampal connectivity to visual-processing regions can predict less food consumption and subsequent weight loss in men needs to be further investigated.

In women, we further evaluated whether the central insulin response in hippocampus FC could be due to hormonal fluctuations during the menstrual cycle. Indeed, animal studies suggest an effect of estrogen signaling on central insulin sensitivity (22). Interestingly, during the luteal phase, characterized by heightened estradiol and progesterone levels, a negative correlation was observed between insulin-mediated hippocampal FC to parts of the visual cortex and estradiol/progesterone ratio. As a result, women with higher estradiol relative to progesterone levels revealed lower hippocampal FC in the luteal phase. Likewise, women with normal menstrual cycles have been reported to exhibit higher reactivity to high-caloric food in the calcarine gyrus in the luteal phase compared to the follicular phase, similar to those using monophasic oral contraception pills (which maintain high progesterone levels) (26). Moreover, during the luteal phase, elevated postprandial insulin levels (triggered by glucose ingestion) were observed to diminish the responsiveness of the calcarine gyrus to food stimuli (26). In addition, behavioral data found no differences in food intake and hippocampus-dependent memories after intranasal insulin administration between men with and without estrogen treatment (23, 25), as well as between postmenopausal and young women (23-25). However, the effect of progesterone was not investigated in these studies. It has been shown that spatial memory is enhanced in the luteal phase, suggesting an interaction between estradiol and progesterone on hippocampal activity (52). Hence, the role of progesterone on hippocampal insulin action is still an open question (25). According to these studies, we speculate that the distinct pattern of hippocampal FC with visual-processing regions could be affected by hormone fluctuations during the menstrual cycle in women. We speculate that this could increase visual processing and food memory in the luteal phase of the cycle. However, in future studies, it will be necessary to explore sex differences in central insulin action within the hippocampus across sexes while controlling for hormonal fluctuations.

Although our study reveals hippocampal FC in response to central insulin over a large age and body weight range, it is essential to recognize certain limitations in our research. In study 1, we did not control for sex hormones in female participants. Hence the role of hormonal fluctuation in aging women still needs to be investigated in future studies. In addition, food intake was not measured in our study. Further research is required to deepen our understanding of how the observed sex differences in central insulin action within the hippocampus correspond to food intake patterns across sexes.

In conclusion, central insulin modulated hippocampal functional connectivity, with sex appearing to play a substantial role. During high-caloric food exposure, central insulin enhanced FC between the hippocampus and inhibitory control region both in men and women. This implies a potential role of the hippocampal network in modulating insulin's anorexic effects. Our findings further support the notion that central insulin action varies between men and women, particularly in terms of the FC observed between the hippocampus and visual-processing regions. This disparity is possibly influenced by sex hormone action.

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Author Contributions

S.X.Z. and S.K. conceptualized and conducted the analysis, and drafted the manuscript; R.V. and L.S. supported the analysis and discussed the results; J.M. and S.K. managed and coordinated the study execution; M.H., H.P., and S.K. designed the study and provided critical review and revisions to the

manuscript; and A.F., A.L.B., H.P., S.K. supervised the work. All authors discussed the results and implications, reviewed and edited the manuscript, and approved its final version.

Disclosures

A.F. reports lecture fees from Sanofi, Novo Nordisk, Eli Lilly, Boehringer Ingelheim, AstraZeneca, and Synlab and serves as a consultant for Novo Nordisk and Sanofi. Outside the present work, M.H. reports research grants from Boehringer Ingelheim and Sanofi to the University Hospital of Tübingen, participation on an advisory board for Boehringer Ingelheim, Sanofi, and Amryt, and lecture fees from Amryt, Sanofi, Eli Lilly, Novo Nordisk, and Boehringer Ingelheim. S.K. reports lecture fees from Novo Nordisk and serves as a consultant for Boehringer Ingelheim. All other authors have no competing interests.

Data Availability

The data are not publicly available due to them containing information that could compromise research participant privacy/consent. The authors will share them by request from any qualified investigator after the completion of a data-sharing agreement.

Clinical Trial Information

Study 1: Effects of Age and Obesity on Brain Insulin Sensitivity (Aging), NCT04372849 (registered November 5, 2020). Study 2: Effect of Central Insulin Administration on Whole-body Insulin Sensitivity in Women, NCT03929419 (registered April 21, 2019).

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3. General Discussion

Pediatric obesity significantly impacts brain development. Maternal metabolic conditions, such as gestational diabetes mellitus (GDM), are considered obesity-promoting factors for the offspring (Hufnagel *et al.*, 2022). GDM exposes the fetus to hyperglycemia and hyperinsulinemia, which alters brain responses to insulin, indicating impairment in central insulin action (Linder *et al.*, 2015; Kullmann *et al.*, 2020a; Hufnagel *et al.*, 2022). Brain Insulin signaling is essential for cognitive function and appetite regulation (Kullmann *et al.*, 2020a). The hippocampus, a critical region for cognition and food intake, is especially vulnerable to adverse prenatal environments and is highly sensitive to insulin (Kanoski and Grill, 2017; Lynch *et al.*, 2019; Kullmann *et al.*, 2020a). Therefore, this thesis systematically reviews neuroimaging studies in children and adolescents with obesity to identify brain alterations and explore the impact of obesity-promoting factors, like GDM, on brain development. It also examines the long-term effects of intrauterine hyperinsulinemia on hippocampal networks in children, as well as the impact of acute intranasal insulin in adults while food cue processing. The main findings of this thesis are summarized in **Figure 4**.

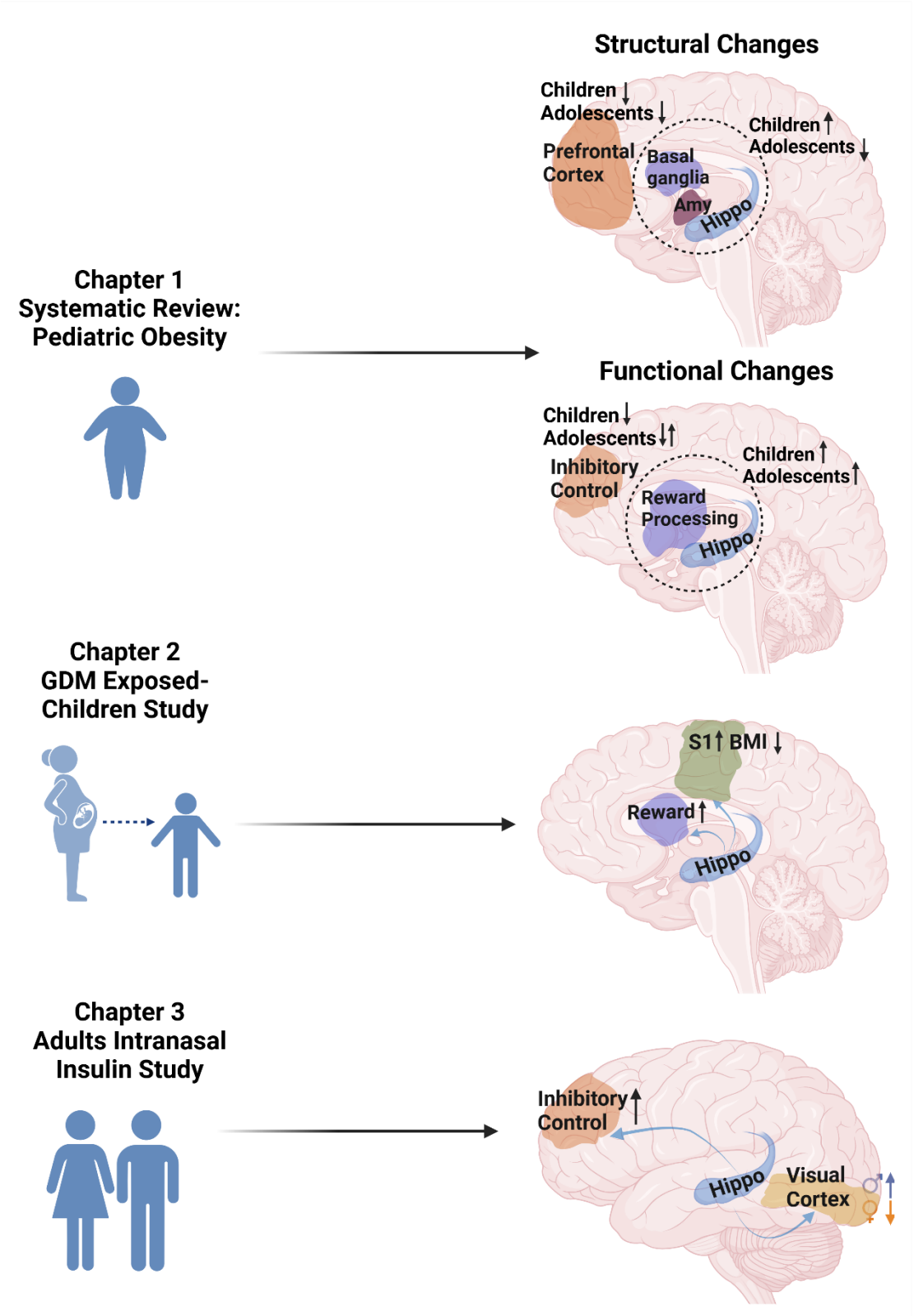

Figure 4

Main findings of the thesis. Amy, amygdala; BMI, body mass index; Hippo, hippocampus; S1, primary somatosensory cortex. Figure was created with BioRender.com.

3.1 Brain and Obesity in Pediatric Population

Obesity arises from multiple factors, including an imbalance between energy intake and expenditure, genetics, environmental influences and psychological factors (Romieu *et al.*, 2017). Food intake, in particular, is regulated by multiple brain regions, including those in the mesolimbic circuity (Devoto *et al.*, 2018). A large body of neuroimaging studies investigated the associations between obesity and brain alterations in both children and adults. Due to the dynamic development of children and adolescents' brain, the impact of obesity on their brains may differ from that in adults, whose brains have already matured. Moreover, pediatric obesity could persist into adulthood, and is linked to a higher risk of metabolic comorbidities (Marcus *et al.*, 2022). Therefore, it is essential to understand the effects of obesity on brain maturation in this population for developing effective interventions to address this public health challenge.

In the literature review of neuroimaging research in recent decade, brain alterations in children and adolescents with obesity were evaluated (**Chapter 1**). Similar to the research in adults, the structure MRI and diffusion tensor imaging (DTI) were applied to detect grey/white matter volumes, cortical thickness and white matter integrity in children. fMRI with diverse paradigms including visual food cue task and tasting to detect neural response to food stimuli (e.g., high-caloric foods and/or low-caloric foods) versus control stimuli (e.g., non-food items) in children and adolescents with obesity. Additionally, cognitive tasks related to food choice, decision-making, attention, and impulse control were conducted, mostly in adolescents, to assess differences in brain regions involved in cognitive processing. Functional connectivity and large-scale brain network analyses were also employed to assess intrinsic brain organization changes during resting-state. Consistent with findings in adults (Li *et al.*, 2023a), mesocorticolimbic system in children and adolescents appears particularly vulnerable to obesity, likely due to their role in reward processing and energy regulation. However, the impact of

obesity on brain structure and function in young populations varies significantly based on developmental stage, particularly in regions like the hippocampus, amygdala and basal ganglia. In children with obesity, these regions often exhibit decreased volumes, whereas adolescents tend to show increased volumes. These opposing patterns suggest that obesity disrupts the typical developmental trajectory, characterized by an inverse “U” shape for volume changes from childhood to adolescence (Herting and Sowell, 2017). The volume and cortical thickness are consistently decreased in the prefrontal cortex.

In response to food-related reward, mesolimbic regions show heightened activation among children and adolescents with obesity relative to their normal-weight counterparts, which also predicts future weight gain. In particular, increased activation of the hippocampus was consistently observed during the processing of high-caloric food cues, palatable food choices, and pleasant tastes in children and adolescents with obesity, and it is related to increased eating in the absence of hunger (Jastreboff *et al.*, 2014; Boutelle *et al.*, 2015; Mestre *et al.*, 2017; Moreno-Padilla *et al.*, 2018). These findings highlight the essential function of the hippocampus in processing food rewards. Furthermore, the correlation between the hippocampal activation and leptin levels indicates an interaction between the hippocampus and endocrine signals during the food reward processing (Jastreboff *et al.*, 2014), which is considered as an advanced regulation (Kanoski and Grill, 2017).

The activation of inhibitory control regions also varies with developmental stage—decreased activation is observed in children, consistent with adults with obesity (Li *et al.*, 2023a), while adolescents exhibit inconsistent activation patterns. Heightened activation in inhibitory regions during adolescence may indicate an effort to exert control over food-related impulses, reflecting the ongoing development of the prefrontal cortex (Moreno-Padilla *et al.*, 2018).

In addition, interventions against pediatric obesity, including exercise, eating

behavioral strategies and weight-loss programs have demonstrated positive effects on both weight management and brain health. While volumetric changes in the hippocampus have not been observed, functional improvements were evident, such as increased activation in inhibitory regions and decreased reward-related brain activity, notably in the hippocampus.

Building on these findings, the hippocampus emerges as a region of interest due to its role in food-related reward processes. Understanding how insulin resistance may influence hippocampal function could offer critical insights into the mechanisms that drive overeating in pediatric populations.

3.2 Hippocampus and Insulin Resistance

The hippocampus regulates food intake by processing interoceptive signals of satiety (e.g., postprandial hormone, insulin) and by inhibiting food-related associative memories (Kanoski and Grill, 2017). In our obesogenic environment, which is rich in external food cues, the hippocampus-dependent regulation helps suppress excessive reward-driven eating (Stevenson *et al.*, 2020). Impaired this regulation can reduce appetite control, leading to overeating even when not hungry (Stevenson *et al.*, 2020).

This region is highly sensitive to adverse factors like insulin resistance, which is often observed in obesity and T2D, contributing to hippocampal dysfunction (Stranahan, 2015). Central insulin resistance, commonly linked to these metabolic conditions, has also been associated with altered hippocampal responses to insulin in both adults and children with obesity. For example, in adults with obesity, absent brain responsiveness to exogenous insulin has been observed, potentially arising from insulin resistance in the hippocampus (Tschritter *et al.*, 2006). Similarly, in children with obesity, an inverse relationship between peripheral insulin sensitivity and mesolimbic reward activity was observed (Adam *et al.*, 2015), alongside heightened activation in the

hippocampus in response to food cues (see **Chapter 1**). This evidence suggests a potential impairment of central insulin action in these children, underscoring a complex interaction between hippocampal function, insulin resistance, and obesity.

Maternal metabolic disturbances, such as GDM, are particularly significant obesity-promoting factors that impact offspring brain development, even independently of the child's current weight status (see **Chapter 1**). GDM exposure leads to fetal hyperinsulinemia, impairing central insulin action as early as fetal development (Kullmann *et al.*, 2020a). This impaired insulin action has been linked to altered hypothalamic inhibition to endogenous insulin (triggered by glucose ingestion) in GDM-exposed children (Page *et al.*, 2019). In addition, GDM-exposed children exhibit structural changes in the hippocampus (Alves *et al.*, 2020; Lynch *et al.*, 2021). Investigating the long-term effects of GDM exposure offers a valuable opportunity to understand how insulin signaling influences hippocampal development and the risk of developing obesity in later stages of life (investigated in **Chapter 2**).

3.3 Long-term Effects of Intrauterine Hyperinsulinemia Exposure on the Hippocampal Network

Exposure to GDM in utero show significant long-term effects on the hippocampus, specifically in how it processes food cues, among children aged 7-11 years. As observed in **Chapter 2** (Zhao *et al.*, 2024a), hippocampal functional connectivity with reward processing regions (e.g., striatum) is higher among children exposed to GDM versus those unexposed, regardless of their current weight status. Consistently, animal studies have shown that perinatal hyperinsulinemia leads to hippocampal insulin resistance in adult offspring, supporting the idea of long-term effects of hyperinsulinemia exposure on brain function (Schmitz *et al.*, 2018). Within the mesolimbic circuitry, hippocampal neurons connect with neurons in

other regions, receiving dopamine signals to convey the rewarding characteristics of external stimuli (Volkow *et al.*, 2011; Kanoski and Grill, 2017). Central insulin plays a key role in modulating neuronal activity and functional connectivity across these regions by suppressing dopaminergic signaling in the striatum (Kullmann *et al.*, 2021). Impaired central insulin action, as is often observed in individuals with metabolic dysfunction, reduces the ability to suppress mesolimbic activity (Tiedemann *et al.*, 2017). Consequently, stronger functional connectivity between the hippocampus and reward regions in children exposed to GDM may be linked to impaired insulin action in the hippocampus. Central insulin resistance has been related to more weight regain after weight loss intervention (Kullmann *et al.*, 2020b). Moreover, the connection of hippocampus-striatum could contribute to food craving (Tomasi and Volkow, 2013). Therefore, the potential impairment of hippocampal insulin action in children with GDM exposure could lead to an increased valuation of food rewards and heightened motivation to eat. These factors may, ultimately, contribute to metabolic disorders, including obesity.

Interestingly, the association between hippocampal functional connectivity and obesity was only observed in children with GDM exposure, specifically involving the hippocampus-somatosensory cortex connection. Similarly, children with obesity show altered hippocampal connection with the somatosensory cortex, a region that plays a role in sensing food properties, such as fat content (De Araujo and Rolls, 2004; Li *et al.*, 2025). This altered functional connections only in GDM-exposed children further support that intrauterine exposure to hyperinsulinemia modifies the development of hippocampal networks, potentially increasing susceptibility to unhealthy eating behaviors.

Taken together, chronic hyperinsulinemia exposure leads to long-lasting changes in hippocampal network during food cue processing, potentially contributing to future weight gain and obesity. Building on these findings in children, we therefore further investigated the response of the hippocampal network to acute central

insulin during a food cue task. The study was conducted in adults in which central insulin was administered by nasal spray (investigated in **Chapter 3**).

3.4 Acute Exogenous Insulin Action in the Hippocampal Network

Acute intranasal insulin enhances functional connectivity between the hippocampus and dorsolateral prefrontal cortex during a visual food cue task (**Chapter 3** (Zhao *et al.*, 2024b)), aligning with findings from resting-state studies in both metabolically healthy and unhealthy adults (Zhang *et al.*, 2015; Kullmann *et al.*, 2017). Evidence suggests that central insulin modulates neural activity in the hippocampus and dorsolateral prefrontal cortex during both food cue processing and resting state (Guthoff *et al.*, 2010; Wagner *et al.*, 2022; Wagner *et al.*, 2023). However, sex differences in insulin-induced neural activity were observed in these regions: women show a decline in hippocampal activity with increasing age, as well as greater dorsolateral prefrontal cortex activation in response to highly desirable food cues compared to men, following intranasal insulin administration (Wagner *et al.*, 2022; Wagner *et al.*, 2023). Interestingly, no sex differences were detected in the functional connectivity between these regions in **Chapter 3** (Zhao *et al.*, 2024b).This consistency could arise from the distinct aspects of brain function: activity reflects the involvement of specific brain region, while functional connectivity represents the synchronization between regions (Kirby *et al.*, 2024).

The dorsolateral prefrontal cortex, a key region for inhibitory control, is involved in regulating appetite, choosing healthier food, and supporting weight management (Weygandt *et al.*, 2013; Weygandt *et al.*, 2015; van Meer *et al.*, 2019; Ester and Kullmann, 2022). We observed the functional connection between the hippocampus and this inhibitory control region is negatively linked to changes in the subjective hunger feeling and food craving. Therefore, individuals with higher functional connectivity show greater reduction in both,

following acute intranasal insulin. This is consistent with resting-state evidence indicating that hippocampal network is predictive of hunger sensations (Kullmann *et al.*, 2017; Hoang *et al.*, 2024). Given the anorexigenic effect of central insulin, the hippocampus-prefrontal cortex connection may increase satiety and suppress the drive for food-related behaviors, contributing to this effect.

3.5 Sex Differences of the Response to Insulin in the Hippocampal Network

During food cue task, acute intranasal insulin action exhibited sex-specific differences in functional connectivity between the hippocampus and the visual processing cortex, with higher functional connectivity in men (**Chapter 3** (Zhao *et al.*, 2024b)). Importantly, no sex differences in peripheral insulin sensitivity were observed, suggesting that central insulin's effect on functional connectivity in the hippocampus is independent of peripheral metabolism. This aligns with the idea that hippocampal insulin resistance may develop independently from peripheral insulin resistance (Fadel and Reagan, 2016; Kullmann *et al.*, 2017). Grillo and colleagues found that rats with selective hippocampal insulin resistance exhibited impaired synaptic plasticity and disrupted hippocampus-dependent spatial learning, despite no impairment in peripheral metabolism (Grillo *et al.*, 2015).
Differences in the hippocampal responsiveness to central insulin between women and men have been noted in the literature (Wagner *et al.*, 2023). Concomitantly, evidence points to a stronger hippocampus-visual cortex connection in men with potential impaired brain insulin action (i.e., mild cognitive impairment), implying a sex-specific response to altered brain insulin levels (Williamson *et al.*, 2022). Neuroimaging studies have demonstrated that the visual processing cortex is involved in food reward processing (Spetter *et al.*, 2020; Avery *et al.*, 2021; Yang *et al.*, 2021; Oren *et al.*, 2022). Additionally, its reactions to both endogenous and exogenous insulin during food cue processing were observed (Guthoff *et al.*, 2010; Kroemer *et al.*, 2013; Heni *et al.*, 2014a). Moreover, only men with higher

functional connection show a greater reduction in food desire following intranasal insulin, aligning with behavioral evidence of central insulin's anorexic effects in men.

Taken together, the response to insulin in the hippocampus during food cue task shows a sex-specific manner. Therefore, we further investigated whether the observed sex differences of hippocampal response to acute insulin could be related to sex hormones in women (investigated in **Chapter 3**).

3.6 Potential Role of Sex Hormones in Sex Differences in Hippocampal Response to Insulin

In women with natural menstrual cycling, we found an inverse relationship between the insulin-mediated hippocampal-visual cortex connection and the estradiol/progesterone ratio, but only in the luteal phase, when both hormones are elevated (**Chapter 3** (Zhao *et al.*, 2024b)). Animal studies have shown the interaction between central insulin and sex hormones, revealing that selectively deletion of brain insulin receptors impacts corpora lutea and antral follicles by reducing luteinizing hormone, which is vital for progesterone and estradiol production (Brüning *et al.*, 2000). In humans, the association between the central insulin sensitivity and peripheral insulin sensitivity is different across the menstrual cycle (Hummel *et al.*, 2023). Therefore, our findings suggest that insulin action in the hippocampus is closely related to sex hormones levels, with hormonal fluctuations throughout the menstrual cycle potentially influencing central insulin effects on the brain.

Evidence from animal studies indicates that estrogen impacts brain insulin sensitivity, with low estrogen levels being crucial for the anorexigenic effect of central insulin (Clegg *et al.*, 2006). However, in humans, no independent effect of estrogen on central insulin action has been demonstrated. For example, no differences in hippocampus-dependent memory or food consumption were

observed between postmenopausal women and young women using estradiol-dominant contraceptives (Krug *et al.*, 2010). Similarly, acute intranasal insulin did not affect food intake in men with or without estradiol treatment (Krug *et al.*, 2018). This inconsistency implies that estrogen may not be the only factor modulating insulin's central effects in humans. The interaction between estradiol and progesterone in increasing hippocampal synaptic density has been demonstrated in animals (Woolley and McEwen, 1993). Moreover, the function of progesterone receptor also relies on activation of estradiol receptor (Lydon *et al.*, 1995). In addition, improvements in spatial memory during the luteal phase in women indicates a potential estradiol-progesterone interaction in hippocampal function in humans (Hussain *et al.*, 2016). Beyond the estradiol/progesterone ratio, we further investigated the effects of estradiol and progesterone separately on insulin-mediated functional connection between the hippocampus and visual cortex during the luteal phase. Our findings suggest that progesterone may act as a primary driver of the observed association between the sex hormones and hippocampal functional connectivity (data not shown). However, the specific role of progesterone in the hippocampal response to central insulin remains unexamined.

3.7 Strength and Limitations of the Studies

This thesis advances our understanding of brain alterations in pediatric obesity, as well as interaction between the hippocampal network and central insulin action in regulating food-related behavior. In addition to providing a comprehensive review of the impact of obesity on brain development, this thesis examines both children with GDM exposure and adults, using fMRI and behavioral assessments to offer a thorough perspective on insulin's impact in the hippocampus across life stages. The studies address significant gaps by suggesting potential impaired insulin action in the hippocampus in humans with intrauterine hyperinsulinemia

exposure. They also investigate how hippocampal responses to central insulin differ by sex, considering the role of sex hormonal action. The findings emphasize the need for individualized approach in future interventions aimed at enhancing central insulin sensitivity.

However, there are limitations that need to be acknowledged. Firstly, a relatively small sample size constrained the statistical power available for subgroup comparisons, especially in examining role of sex hormones in premenopausal female adults. Future studies with a larger sample size in women with natural menstrual cycle should be conducted to detect the differences of insulin-mediated hippocampal functional connectivity between the follicular and luteal phases. This is important as hormonal fluctuations across these phases can significantly impact brain function and insulin sensitivity (Hummel *et al.*, 2023). Secondly, although we interpreted our results in relation to eating behaviors, we did not include behavioral questionnaires, such as those assessing food cravings (in the GDM study, **Chapter 2**), or direct measures of energy intake. This limitation leaves the relationship between hippocampal functional connectivity alterations and metabolic processes in children unexamined. It also restricts our understanding of sex differences in hippocampal insulin action and their influence on energy intake in adults. Finally, the cross-sectional design of current studies restricts our capacity to assess whether the observed functional differences in the hippocampus may be linked to future weight gain and development of metabolic conditions in both children and adults.

3.8 Conclusions and Future Directions

The influence of pediatric obesity on brain structure and function, particularly in the hippocampus, amygdala and basal ganglia within the mesocorticolimbic circuitry, varies significantly based on developmental stage in pediatric populations. Intrauterine GDM exposure (i.e., hyperinsulinemia exposure), as an

obesity-promoting factor, leads to long-lasting effects on the hippocampal network. These changes may increase the risk of future weight gain and metabolic disorders. Importantly, acute insulin demonstrates sex-specific differences in hippocampal network response, potentially influenced by sex hormones. These findings highlight the importance of early interventions for adverse prenatal exposures, such as GDM, due to their long-term effects on brain development. Moreover, individualized approaches for addressing conditions involving central insulin resistance, such as obesity and type 2 diabetes, that account for sex-specific responses may provide additional benefits.

Future studies could utilize intranasal insulin administration in children exposed to GDM to investigate and provide direct evidence of hippocampal insulin resistance in this population, as such evidence is still lacing. Additionally, the interaction between central insulin and progesterone in the brain warrants further exploration, and studies comparing the effects of central insulin between young women on progesterone-dominant contraceptives and postmenopausal women could be particularly informative. Given the distinct roles of hippocampal subregions, it remains unclear which subregions are responsible for the observed insulin-induced changes in functional connectivity in children and adults. Future research using high-field MRI, which allows for precise hippocampal segmentation, could address this knowledge gap.

Behavioral data in adults indicate that the effects of central insulin on food intake differ between sexes depending on nutritional states (Benedict *et al.*, 2008; Hallschmid *et al.*, 2012). The hippocampus controls food intake by sensing physiological hunger and satiety signals (Kanoski and Grill, 2017). Therefore, future research could benefit from exploring sex differences in central insulin action during different nutritive states, particularly in the hippocampus, to understand the neural mechanisms behind these behavioral differences. Moreover, comparing central insulin action between individuals with normal

weight and those with obesity across different nutritive states could provide further insights into central insulin resistance in obesity.

4. Summary

Pediatric obesity significantly impacts brain development, with GDM being one of the contributing factors. GDM exposes the fetus to hyperinsulinemia, which alters brain responses to endogenous insulin and suggests impaired central insulin action. Insulin signaling in the brain plays a crucial role in cognitive function and appetite regulation. The hippocampus, a critical region for cognition and food intake, is particularly vulnerable to adverse prenatal environments and is highly sensitive to insulin. However, the response of hippocampal network to chronic hyperinsulinemia exposure (from GDM) and acute exogenous insulin during food cue processing remains unclear.

Firstly, this thesis systematically reviews neuroimaging studies in children and adolescents with obesity to identify brain alterations and explore the impact of obesity-promoting factors, like GDM, on brain development. The influence of obesity on brain structure and function, particularly in the hippocampus, amygdala and basal ganglia within the mesocorticolimbic circuitry, varies cross developmental stage. Maternal obesity and GDM influence brain development independent of the child's current weight status.

Secondly, fMRI was used to assess hippocampal network during a food cue task in children aged 7–11 years, comparing those exposed to GDM with those not exposed. Associations between BMI and hippocampal network were analyzed in both groups. Children with GDM exposure showed stronger functional connectivity between the hippocampus and reward processing regions. An association between the adiposity and hippocampal functional connection to the somatosensory cortex was only observed in GDM group. These changes suggest that intrauterine GDM exposure leads to long-lasting effects on the hippocampal network, potentially increasing the risk of future weight gain and metabolic disorders. This highlights the importance of early interventions for adverse prenatal exposures due to their long-term effects on brain development.

Thirdly, the response of hippocampal network to intranasal insulin during food cue processing was explored in adults using fMRI. The influence of sex hormone levels on hippocampal functional connectivity was assessed in premenopausal women. Central insulin increased hippocampal functional connectivity with the inhibitory control region, supporting insulin's anorexic effect. Sex differences were observed in the functional connectivity between the hippocampus and visual cortex, potentially related to sex hormones fluctuation in women. These findings suggest that individualized interventions for central insulin resistance conditions, accounting for sex-specific responses, may provide additional benefits.

5. German Summary

Adipositas im Kindes- und Jugendalter beeinflusst die Gehirnentwicklung erheblich, wobei Gestationsdiabetes mellitus (GDM) zu den beitragenden Faktoren zählt. GDM führt zu einer fetalen Hyperinsulinämie, die die Gehirnantwort auf endogenes Insulin verändert und auf eine gestörte zentrale Insulinwirkung hinweist. Insulinsignalwege im Gehirn spielen eine entscheidende Rolle bei kognitiven Funktionen und der Appetitregulation. Der Hippocampus, eine Schlüsselregion für Kognition und Nahrungsaufnahme, ist besonders anfällig für ungünstige pränatale Umwelteinflüsse und hochsensibel gegenüber Insulin. Die Reaktion des hippocampalen Netzwerks auf chronische Hyperinsulinämie-Exposition (durch GDM) und akutes exogenes Insulin während der Verarbeitung von Nahrungsreizen ist jedoch noch unklar.

Erstens bietet diese Dissertation eine systematische Übersicht über neuroimaging Studien zu Gehirnveränderungen bei adipösen Kindern und Jugendlichen, um diese Veränderungen zu identifizieren und die Auswirkungen von Adipositas-fördernden Faktoren wie GDM auf die Gehirnentwicklung zu untersuchen. Der Einfluss von Adipositas auf die Gehirnstruktur und -funktion, insbesondere im Hippocampus, in der Amygdala und den Basalganglien innerhalb der mesokortikolimbischen Schaltkreise, variiert je nach Entwicklungsphase. Mütterliche Adipositas und GDM beeinflussen die Gehirnentwicklung unabhängig vom aktuellen Gewicht des Kindes.

Zweitens wurde fMRT verwendet, um das hippocampale Netzwerk während einer Nahrungsreizaufgabe bei Kindern im Alter von 7–11 Jahren zu bewerten. Hierbei wurden Kinder mit GDM-Exposition mit solchen ohne Exposition verglichen. Die Assoziationen zwischen BMI und dem hippocampalen Netzwerk wurden in beiden Gruppen analysiert. Kinder mit GDM-Exposition zeigten eine stärkere funktionelle Konnektivität zwischen dem Hippocampus und Belohnungsverarbeitungsregionen. Eine Assoziation zwischen Adipositas und

der hippocampalen funktionellen Verbindung zum somatosensorischen Kortex wurde nur in der GDM-Gruppe beobachtet. Diese Veränderungen weisen darauf hin, dass die intrauterine GDM-Exposition zu langfristigen Effekten auf das hippocampale Netzwerk führt und möglicherweise das Risiko für zukünftige Gewichtszunahme und Stoffwechselerkrankungen erhöht. Dies unterstreicht die Bedeutung früher Interventionen bei ungünstigen pränatalen Expositionen aufgrund ihrer langfristigen Auswirkungen auf die Gehirnentwicklung.

Drittens wurde die Reaktion des hippocampalen Netzwerks auf intranasales Insulin während der Verarbeitung von Nahrungsreizen bei Erwachsenen mittels fMRT untersucht. Der Einfluss von Sexualhormonspiegeln auf die hippocampale funktionelle Konnektivität wurde bei prämenopausalen Frauen bewertet. Zentrales Insulin erhöhte die funktionelle Konnektivität des Hippocampus mit der Region der inhibitorischen Kontrolle und unterstützte die anorektische Wirkung von Insulin. Geschlechtsspezifische Unterschiede wurden in der funktionellen Konnektivität zwischen Hippocampus und visuellem Kortex beobachtet, die möglicherweise mit Hormonschwankungen bei Frauen zusammenhängen. Diese Ergebnisse deuten darauf hin, dass individualisierte Interventionen für zentrale Insulinresistenz, die geschlechtsspezifische Reaktionen berücksichtigen, zusätzliche Vorteile bieten könnten.

6. References

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7. Declaration of Contribution of Others

For **Chapter 1**, Sixiu Zhao (candidate) was responsible for the majority of the work, including method selection (90%), data acquisition (90%), data analysis (85%), results interpretation (70%), and manuscript writing (100%). Hubert Preissl contributed to conceptualizing the research (50%) and reviewing the manuscript (10%). Stephanie Kullmann also contributed to conceptualizing the research (50%), data analysis (15%), results interpretation (25%), and manuscript review (70%). Lorenzo Semeia assisted with method selection (10%), data acquisition (10%) and manuscript review (10%). Ralf Veit contributed by reviewing the manuscript (5%), while Julia Moser supported results interpretation (5%) and manuscript review (5%).

For **Chapter 2**, Sixiu Zhao (candidate) was responsible for method selection (60%), data analysis (70%), results interpretation (70%), and manuscript writing (100%). Stephanie Kullmann contributed to conceptualizing the research (30%), method selection (20%), results interpretation (30%), and manuscript review (45%). Hubert Preissl conceptualized the research (10%) and reviewed the manuscript (20%). Kathleen A. Page and Anny H. Xiang each contributed to conceptualizing the research (30%) and reviewing the manuscript (10%). Lorenzo Semeia analyzed data (15%) and reviewed the manuscript (5%), while Ralf Veit contributed to method selection (20%), data analysis (15%), and manuscript review (5%). Shan Luo, Brendan C. Angelo, and Ting Chow equally shared data collection responsibilities (33.3% each). Andreas L. Birkenfeld reviewed the manuscript (5%).

For **Chapter 3**, Sixiu Zhao (candidate) carried out method selection (70%), data analysis (75%), results interpretation (70%), and manuscript writing (100%). Stephanie Kullmann contributed to conceptualizing the research (70%), method selection (10%), data collection (45%), results interpretation (30%), and manuscript review (45%). Hubert Preissl conceptualized the research (20%),

interpreted results (10%), and reviewed the manuscript (10%). Martin Heni contributed to conceptualizing the research (10%), data collection (25%), and manuscript review (5%). Ralf Veit contributed to method selection (20%), data collection (10%), data analysis (15%), and manuscript review (2%). Lorenzo Semeia analyzed data (5%) and reviewed the manuscript (2%). Julia Hummel participated in data collection (10%), data analysis (5%), and manuscript review (2%). Andreas Fritsche collected data (10%) and reviewed the manuscript (2%). Andreas L. Birkenfeld and Leontine Sandforth each reviewed the manuscript (5% and 2%, respectively).

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"路漫漫其修远兮，吾将上下而求索"。