Effects of gestational thyroid hormone exposure: insights into neurodevelopment using MRI

Thesis submitted in accordance with the requirements of Cardiff University for the degree of Doctor of Medicine (MD)

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Abstract

Introduction:

Children exposed to gestational thyroid abnormalities may face an increased risk of adverse neurodevelopmental outcomes.

Aim:

To investigate if abnormal maternal thyroid hormone (TH) bioavailability *in utero* affects child brain development as assessed with neuroimaging.

Methods:

A systematic review was conducted to identify MRI neuroimaging studies of participants exposed to thyroid function abnormalities *in utero* or during childhood. The Controlled Antenatal Thyroid Screening (CATS) III study follows a subset from the original CATS study cohort, a randomised controlled trial examining effects of maternal gestational thyroid function (GTF) on offspring development. A total of 85 children aged 11 to 16 underwent T1-weighted structural MRI. Participants were divided into groups: untreated suboptimal GTF (SGTF)(n=21), normal GTF (n=24), optimally treated SGTF (n=20), and overtreated SGTF (n=20). The primary outcome examined the association of SGTF and its treatment with global brain volumes. Secondary outcomes investigated the association of maternal thyrotropin (TSH) and free thyroxine (fT4) levels with brain volumes.

Results:

The systematic review included 22 studies on maternal thyroid dysfunction (MTDF) during pregnancy, congenital hypothyroidism (CH), and transient hypothyroxinemia of prematurity (THOP). The heterogeneity in study design made it difficult to extrapolate or combine findings with confidence. The foetal brain appears to be most sensitive to thyroid abnormalities in the first trimester, but CH and THOP studies also indicated long-term impacts on brain morphology. TSH had the most consistent association with reported differences.

The CATS III study found no significant effect of treating mild maternal thyroid dysfunction on brain morphology at a macrostructural level. A weak correlation between first trimester maternal TSH levels and several grey matter regional volumes was noted but did not remain significant after correction for multiple testing.

Conclusion: Macrostructural neuroimaging results of the effect of mild gestational thyroid function abnormalities are often weak and conflicting. Other MRI modalities are the next step to investigate potential microstructural effects of thyroid function on brain development.

"Quis tumidum guttur miratur in Alpibus?"

Who wonders at a swollen throat in the Alps?

Juvenal, Satire 13, line 162 (100-127 AD)

"I am satisfied. I have seen the principal features of Swiss scenery — Mount Blanc and the goiter— and now for home."

Mark Twain, 1880

"Simple goitre is so simple to prevent that it can disappear as soon as society makes the choice."

David Marine, 1915, as quoted in Chance and Commitment: Memoirs of a Medical Scientist (2005), Basil S. Hetzel.

"Science is the systematic classification of experience".

George Henry Lewes, 1877, The Physical Basis of Mind.

"Human rights are not things that are put on the table for people to enjoy. These are things you fight for and then you protect."

Wangarĩ Maathai (1940–2011)

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As I have been finishing this thesis, I have increasingly thought about Sheila Q and her daughter, and how thyroid disease can have life long impacts on people and families. Dr. Leaver for teaching me about compassionate medicine.

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Marie deserves a whole line to herself. You and Ben are just awesome, but you particularly.

And of course, my Mum.

Dedication

I dedicate this thesis in memory to George and Gwen Milner.

Abbreviations

AD	axial diffusivity
ADHD	attention deficit hyperactivity disorder
ASD	autism spectrum disorder
CH	congenital hypothyroidism
DIO	deiodinase
DTI	diffusion tensor imaging
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
fT3	free T3
fT4	free T4
HYPO	overt hypothyroidism (term used by Canadian group)
IQ	intelligence quotient
MD	mean diffusivity
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MTDF	maternal thyroid dysfunction
qMT	quantised magnetisation transfer
RD	radial diffusivity
RTH	resistance to thyroid hormones
rT3	reverse T3
SGTF	suboptimal gestational thyroid function
T3	triiodothyronine
T4	thyroxine

TFTs	thyroid function tests
TH	thyroid hormone
THOP	transient hypothyroxinaemia of prematurity
THR	thyroid hormone receptor
TSH	thyroid stimulating hormone
VST	visuospatial memory tasks
VAM	verbal associative memory tasks

Publications and presentations from this work

Publication: Anna Scholz, Carolyn B McNabb, Laura Bloomfield, Raghav Bhargava, Charlotte Hales, Colin M Dayan, Peter N Taylor, John H Lazarus, Onyebuchi Okosieme, Marian Ludgate, Derek K Jones, D Aled Rees, Controlled Antenatal Thyroid Screening Study III: Effects of Gestational Thyroid Status on Adolescent Brain Morphology, *The Journal of Clinical Endocrinology & Metabolism*, 2024;, dgae338, https://doi.org/10.1210/clinem/dgae338

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Chapter 1

Chapter 1. Introduction

1.1 The thyroid

Iodine and thyroid hormones play an essential role throughout the living world. In all vertebrates, including humans, the thyroid gland is a universal feature, with thyroid hormones almost the sole use of iodine in the body (Stenzel & Huttner, 2013). While invertebrates lack thyroid glands, iodine-based thyroid hormone precursors are present across the kingdom (Dumont et al., 2011). Iodine has been identified in organisms dating back as far as 3 billion years (Venturi et al., 2000). The evolutionary significance of thyroid function is evident in key processes such as metamorphosis in amphibians, as noted by Furlow & Neff (2006). In the developing brain, thyroid hormones play a crucial role in processes like neuronal differentiation, migration, myelination, and the formation of intraneuronal connections. The timing and rhythm of hormone production exhibit species-specific variations (Crockford et al., 2003).

1.1.1 The thyroid gland: anatomy and development

The mature thyroid is a butterfly shaped gland situated in the lower anterior section of the neck, in front of the trachea (Figure 1.1). It both produces and stores thyroid hormones. The thyroid is the first endocrine gland to develop in the foetus, beginning in the third week of gestation. It forms from the pharyngeal pouches, starting at the base of the tongue and migrating along the midline over the next four weeks down to the lower neck and creating two lobes, the left and right, connected by the isthmus. The thyroid gland starts to produce thyroid hormones during the 12th week of gestation (Rosen and Sapra, 2019). Thyroid hormones are stored in follicles within pseudolobules in the thyroid, released into the blood stream as needed. (Calzà, 2018)

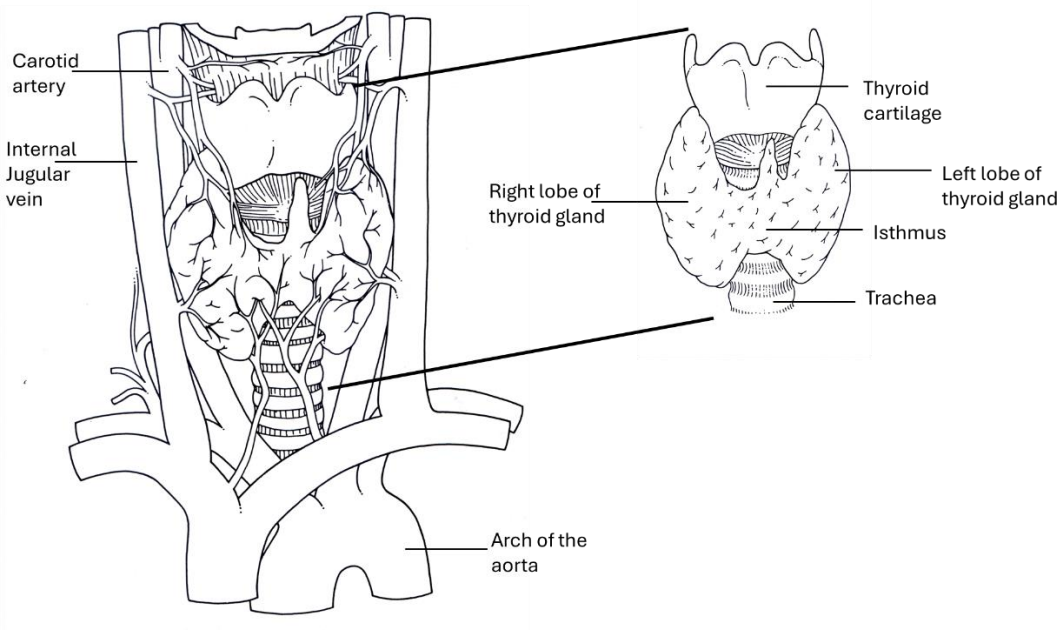


Figure 1.1. Anatomy of the thyroid gland and the surrounding structures in the neck. (Adapted from The Sourcebook of Medical Illustration, The Parthenon Publishing Group, P. Cull, ed., 1989).

1.2 Thyroid hormones: structure and actions

The nomenclature of 3,3′,5-triiodothyronine (T3) and 3′,5′,3,5-tetraiodo-L-thyronine (T4 or L-thyroxine) refers to the number of iodine atoms incorporated into each structure, three and four respectively (Figure 1.2). The thyroid is the only use of iodine in the human body. The stimulant for production of thyroid hormones is thyroid stimulating hormone (TSH), which is released by the anterior pituitary gland in response to levels of circulating free T4 and T3 and other signals of metabolism. In humans, T4 accounts for 80% of the thyroid hormones produced by the thyroid gland (Maia et al., 2005). Within the thyroid gland and peripheral tissues, deioidinases (DIO) can cleave an iodine atom from the ring of iodine atoms in T4 to form a smaller ring, either T3 or reverse T3 (rT3). The thyroid gland is the only source of T4 in the body.

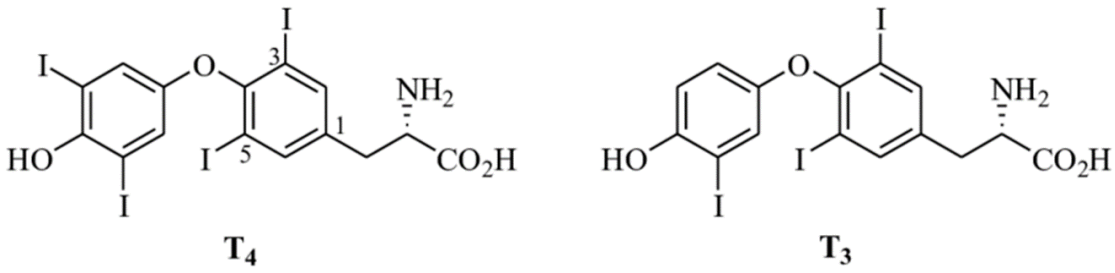


Figure 1.2. Structure of thyroid hormones. T4, 3,3′,5,5′-tetraiodothyronine or thyroxine; T3, 3,3′,5-triiodothyronine or triiodothyronine; (de Castro et al. 2015)

T3 is the active form of TH that has a number of genomic actions, controlling a variety of transcription pathways. Reverse T3 was previously believed to be an inactive metabolite, but has been found to have non-genomic effects, as can T4 (Davis et al., 2016). For example, T4, and to a lesser extent T3 and rT3, can bind to integrin αvβ3 (Davis et al., 2019). Integrin αvβ3 is a cell membrane receptor that plays a crucial role in cellular adhesion and signalling, particularly in the context of angiogenesis, bone metabolism, and tumour progression.

The genomic actions of T3 are exerted by acting through nuclear receptors, which are part of the steroid/nuclear receptor superfamily of ligand-inducible transcription factors (Moran and Chatterjee, 2015). T3 can also affect mitochondrial function. Thyroid hormone in humans affects metabolic regulation, normal growth and development, regulation of body weight, lipid metabolism, cardiovascular function, reproduction and bone health.

More recently, the observation in terminal cancer patients of an association of reduced FT4 levels with increased life-expectancy suggests that T4 not just simply a pro-hormone, but may be a hormone that can have non-genomic actions (Hercbergs et al., 2019).

Thyroid hormones play a key role in neurodevelopment during gestation and infancy. The detrimental effect of poor thyroid function on intelligence and behaviour has been recorded over centuries, via the association between endemic goitre and cretinism (Zimmermann, 2008).

1.3 Regulation of thyroid function

The hypothalamic-pituitary-thyroid (HPT) axis regulates thyroid hormone production and secretion The hypothalamus releases thyrotropin-releasing hormone (TRH) to stimulate production of thyroid stimulating hormone (TSH) from the anterior pituitary. T4 and T3 have an inhibitory influence on the production of TSH. The majority of thyroid hormones produced are in the form of T4, which has a longer half-life than T3, acting as a thyroid hormone reservoir that can be converted into the more active T3 when required.

Once thyroid hormone levels reach a certain threshold, they send negative feedback signals to the hypothalamus and pituitary gland, inhibiting the further release of TRH and TSH (Figure 1.3). This feedback mechanism helps maintain thyroid hormone concentrations within a narrow physiological range. Various factors, including stress, illness, medications, and environmental factors, can influence the HPT axis.

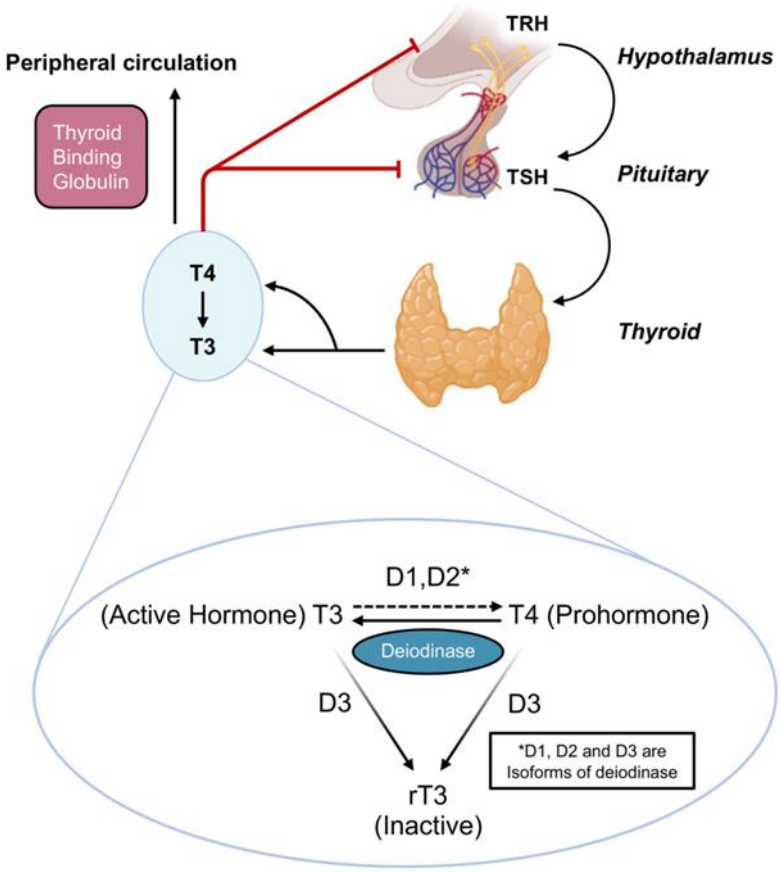


Figure 1.3. Hypothalamic Pituitary Thyroid Axis. Interactions between TRH, TSH, T3, and T4 along the hypothalamic pituitary thyroid axis are shown alongside their transport by thyroxine binding globulin and conversion by deiodinase 1, 2, and 3. Abbreviations: TRH, thyrotropin releasing hormone; TSH, thyroid stimulating hormone; T4, thyroxine; T3, triiodothyronine; rT3, reverse T3; D, deiodinase. Diagram reproduced from Brown et al. (2023).

Peripheral regulation

Ninety-nine percent of thyroid hormones circulating in the blood are bound to serum proteins, such as thyroid hormone binding globulin (TBG). Only free T3 or T4 can move across into target tissues, using thyroid hormone transporters. T4 has a lower affinity for nuclear thyroid hormone receptors (THR) than T3, so mostly acts as a pro-hormone, with deiodinase enzymes in peripheral tissues cleaving it to the high affinity T3 or reverse T3 (rT3) (van der Spek et al, 2017).

Peripheral regulation of thyroid hormone action involves molecular mechanisms that modulate the responsiveness of target tissues to thyroxine (T4) and

triiodothyronine (T3). Key components in this regulation include thyroid hormone transporters, deiodinases, and thyroid hormone receptors.

Thyroid hormone transporters, such as monocarboxylate transporter 8 (MCT8) and organic anion transporter 1C1 (OATP1C1), facilitate the entry of T4 and T3 into cells, influencing their local availability (Mayerl et al., 2014; Moran et al., 2022). The differential expression and activity of these transporters contributes to tissue-specific variations in thyroid hormone uptake, influencing the responsiveness of target organs.

Deiodinases, specifically types 1 and 2 (DIO1 and DIO2), play a crucial role in regulating thyroid hormone action at the peripheral level. DIO1 converts T4 to the more biologically active T3, enhancing the availability of the active hormone within target tissues. DIO2, localized in specific tissues, further amplifies local T3 concentrations, providing a fine-tuned control mechanism for thyroid hormone responsiveness (Galton et al., 2014).

The actions of thyroid hormones are mediated through thyroid hormone receptors (THRs), which bind to specific response elements on target genes (Bernal, 2015). The expression of TR isoforms, TRα and TRβ, varies across tissues, contributing to the tissue-specific effects of thyroid hormones. Additionally, coactivators and corepressors modulate the transcriptional activity of TRs, further refining the regulation of thyroid hormone responsive genes (Zuñiga et al., 2022).

Deiodinases

Deiodinases are enzymes that regulate the conversion of the prohormone T4 to the biologically active T3, ensuring that tissues receive an appropriate amount of active thyroid hormone. There are three main types of deiodinases: type 1 (DIO1), type 2 (DIO2), and type 3 (DIO3). The different types help to regulate local thyroid hormone levels in specific tissues and cells.

Type 1 Deiodinase (DIO1) is primarily found in the liver, kidney, and thyroid gland, maintaining the body's metabolic rate. Type 2 Deiodinase (DIO2) is predominantly located in the brain, thyroid gland, and skeletal muscle. It acts in a similar manner to DIO1, but is particularly significant in the brain, where it contributes to the regulation of T3 levels in neural cells. Type 3 Deiodinase

(DIO3) is found in the placenta, brain, and other tissues. DIO2 and DIO3 have opposite actions and help modulate cellular exposure and response to thyroid hormones (Galton et al., 2014). DIO2 is present in the endoplasmic reticulum, and converts T4 to T3 for nuclear import, so modulating activity (Arrojo e Drigo and Bianco, 2011). DIO3 is present on the plasma membrane, inactivates thyroid hormones, and is thought to protect tissues from excess T3 (Arrojo e Drigo, 2011). The expression of the different enzymes has been found to occur at specific time points in foetal development, helping the body control local actions of THs during neurodevelopment (Chan et al., 2002).

Thyroid hormone receptors

Thyroid hormone receptors (THRs) are nuclear receptors that mediate the biological effects of THs. In the absence of thyroid hormones, THRs are typically bound to corepressors, maintaining a repressive state. Thyroid hormones act as ligands to activate THRs, and binding of T3 modulates gene transcription/expression. THRs exert their influence on gene expression by binding to specific thyroid hormone response elements (TREs) within the regulatory regions of target genes. Upon thyroid hormone binding, conformational changes occur, facilitating the dissociation of corepressors and the recruitment of coactivators. This dynamic interplay modulates chromatin structure, promoting the initiation of transcription and subsequent mRNA synthesis. The resultant proteins contribute to various physiological processes, including metabolism, growth, and development (Koenig, 1998).

THRs exist in two isoforms, TRα and TRβ, each encoded by distinct genes located on different chromosomes (Moran et al., 2022). TRα and TRβ have various isoforms, with beta 1 and beta 2, along with alpha 1 and alpha 2, playing distinct roles. The tissue-specific distribution of these isoforms contributes to the diverse physiological responses elicited by THs.

TRβ1 regulates metabolic genes and impacts growth. TRβ2 is primarily found in the hypothalamus and pituitary gland, contributing to HPT axis regulation. TRα1 is widely distributed in the body, including the central nervous system, and influences cardiac function, skeletal development, and metabolism (Moran and Chatterjee, 2015). The role of TRα2 is more of a mystery, but unlike other THRs,

does not bind T3 and evidence suggests TRα2 may have inhibitory effects on THRbeta1 (Hönes et al., 2022). TRα2 is predominantly expressed in brain and heart tissues, but a lack of isoform-specific antibodies and very few human cases identified, has complicated investigation. Together, these isoforms orchestrate the diverse effects of thyroid hormones, showcasing the intricate regulatory mechanisms governing physiological processes across various tissues and organs (Liu et al., 2019; Wejaphikil et al., 2019).

Thyroid hormone transporters

Thyroid hormone (TH) transporters regulate the intracellular availability of thyroid hormones. Facilitating the passage of these hormones across cellular membranes, thyroid hormone transporters play a pivotal role in maintaining physiological thyroid hormone levels and orchestrating the diverse actions of THs. In humans, the predominant thyroid hormone transporter, of both T4 and T3 across the blood brain barrier and into neuronal and glial cells, is monocarboxylate transporter 8 (MCT8). A second thyroid hormone transporter is organic anion transporter 1C1 (OATP1C1) which is specific for T4 transport across the blood brain barrier and into astrocytes (Bernal, 2018; Moran et al. 2022). In mice OATP1C1 is the predominant thyroid hormone transporter, but in humans and apes it is expressed at very low levels (Mayerl et al., 2012).

In humans, MCT8 transports both T4 and T3 across the blood brain barrier. Unlike in peripheral tissues, or the rodent brain, there are no significant alternative transporters for thyroid hormones in the blood brain barrier in primates, rendering them wholly dependent on functioning MCT8 to allow thyroid hormones to reach the brain tissue. Abnormal MCT8 function is reflected by increased free T3, low free T4, and normal TSH levels in the serum. Therefore, the brain experiences severe hypothyroidism whilst the rest of the body is exposed to hyperthyroidism due to higher T3 (Moran et al., 2022).

1.4 Clinical diseases (non-malignant)

Table 1.1 provides a summary of diseases of the thyroid, not including cancer.

Goitre

A goitre is an enlargement of the thyroid gland, evident as a swelling in the neck. Common aetiologies include iodine deficiency, autoimmune thyroid disorders (such as Hashimoto's thyroiditis and Graves' disease), nodular thyroid disease, thyroiditis, genetic factors, certain medications (e.g. lithium, amiodarone), pregnancy-related hormonal changes, and endemic goitre in regions with low iodine levels. Treatment of the underlying cause can help, but surgery may be required if there is either significant retrosternal extension causing tracheal compression or cosmetic concerns.

Hypo- and hyperthyroidism

In hypothyroidism, there is insufficient production of thyroid hormones T3 and T4, while hyperthyroidism involves excessive production. Primary hypo- or hyperthyroidism indicates that the dysfunction originates within the thyroid gland itself. Secondary hypothyroidism arises from issues outside the thyroid gland, commonly due to pituitary failure, resulting in inadequate production of TSH to stimulate further thyroid hormone production. Overt hypo- or hyperthyroidism is defined by clinical symptoms and/or, for primary conditions, biochemically, with both components of the axis exhibiting abnormalities. Subclinical hypo- or hyperthyroidism is characterised by alterations in TSH while FT4 levels remain within the normal range, such as normal T4 with raised TSH (subclinical hypothyroidism) or normal T4 with suppressed TSH (subclinical hyperthyroidism).

Hypothyroidism manifests clinically as fatigue, weight gain, cold intolerance, and constipation, with laboratory findings of elevated thyroid-stimulating hormone (TSH) and decreased free thyroxine (fT4). Primary hypothyroidism, originating in the thyroid gland, commonly results from Hashimoto’s disease (an autoimmune thyroiditis), viral thyroiditis, iatrogenic or extreme iodine deficiency. Secondary hypothyroidism stems from hypothalamic/pituitary dysfunction, leading to insufficient TSH production.

In contrast, hyperthyroidism is characterised by symptoms such as weight loss, heat intolerance and palpitations, accompanied by decreased TSH and elevated fT4 and T3 levels. Graves' disease, an autoimmune disorder, represents a prevalent cause of primary hyperthyroidism, marked by stimulatory thyrotropin receptor antibodies. Autonomous nodules or toxic multinodular goitres are additional aetiologies, characterised by thyroid autonomy and excessive hormone production independent of TSH regulation.

Iodine deficiency and thyroid dysfunction in early childhood: cretinism and congenital hypothyroidism

The link between thyroid status and neurodevelopment has long been recognised, ever since the first descriptions of the association of endemic goitre and neurological cretinism in the Middle Ages (Zimmermann, 2008), followed by thyroid aplasia and severe learning difficulties (Curling, 1850). In the 19th century, the importance of iodine was appreciated, and eventually recognised as a component of thyroid hormones themselves (Zimmermann, 2008). The last half of the 20th century saw the almost complete eradication of neurological cretinism, the most common cause of preventable intellectual disability in the world, mainly via salt iodinisation programmes and the ability to diagnose and promptly treat mothers with overt hypothyroidism (Querido, 1975; WHO).

The term cretin was used to describe those individuals with neurological, intellectual, and audiometric deficits (Boyages, 1988). The word is thought to have been derived from the Swiss French dialect and was first introduced to the medical literature in the 18th century. Neurological cretinism results from severe iodine deficiency during gestation, leading to inadequate maternal thyroid hormone production and impeding normal brain development. Cretinism is characterised by profound and irreversible intellectual disability, developmental delays, and neurological deficits. When adequate iodine was provided before pregnancy or in the first half of pregnancy, neurological cretinism was prevented (Choufer et al., 1965; Pharoah and Connelly, 1991; Cao et al., 1994), but not if given later in pregnancy or to the infant after birth.

In contrast, congenital hypothyroidism (CH), previously called myxoedematous cretinism, occurs when the child itself is hypothyroid after birth, due to severe

iodine deficiency in early childhood or due to congenital hypothyroidism because of thyroid dysgenesis or enzyme deficiencies (Curling, 1850; Morreale de Escobar, 2004). Children with CH are able to receive maternal thyroid hormones during gestation, so are protected from extreme thyroid hormone deficiency until after birth. Due to national screening programmes, CH can now be detected at birth, allowing for levothyroxine treatment to be promptly initiated and significant neurological sequelae avoided.

For a more in-depth summary of the history of discovery and recognition of the importance of iodine and quotes illustrating the association of endemic goitre and cretinism, please see appendix 7.1.

Genetic disorders affecting thyroid function and neurodevelopment

Dysregulation of thyroid hormone receptor or transporter function can lead to a spectrum of rare clinical disorders. These have helped improve our understanding of the physiological functions of these receptors and transporters in humans. This knowledge has further emphasised the importance of the thyroid in early life and in neurodevelopment.

Thyroid hormone resistance

Resistance to thyroid hormone beta (RTHβ) is the commonest form of thyroid hormone receptor resistance, occurring in 1 in 19,000-40,000 of the population (LaFranchi et al., 2004; Vela et al., 2019). RTHβ is characterised as a central resistance to thyroid hormone, whereby the mutant receptor exerts a dominant-negative action on the wild-type receptor, leading to the characteristic biochemical hallmark of non-suppressed or raised TSH due to the impaired response of THRβ to circulating thyroid hormones, with raised fT4 and fT3. Those affected experience a relative hypothyroidism in tissues where THRβ dominates and hyperthyroidism in THRα sites, particularly cardiac and brain tissues. Different mutations have different effects on receptor function. This, and the differences in THRβ expression among organs, makes understanding the relationship between genotype and phenotype in patients difficult. Gene manipulation techniques have helped delineate some aspects in mice. Both homozygous patients and mice exhibit more severe phenotypes, including a large

goitre, hearing and visual impairment and dysregulation of the hypothalamic-pituitary-thyroid axis. Those affected often display an attention deficit and hyperactivity disorder (ADHD) like phenotype of behaviour (Uter et al., 2020).

Initially, THRα mutations were presumed to be lethal *in utero*, since no human cases were identified for many years. However, mouse models demonstrated that mutations in THRα were not lethal. The first human case was described in 2012 (Bochukova et al., 2012), with 40 patients identified worldwide by 2021 (Erbaş & Demir, 2021). The main clinical signs of RTHα include growth retardation and gastrointestinal dysfunction such as severe constipation. Speech and motor milestones are also delayed, with variable degrees of reduced IQ.

THRβ predominates in the pituitary gland, hence the hypothalamic-pituitary-thyroid axis is minimally affected in patients with RTHα. In the absence of a severe biochemical signature, patients with RTHα are thus hard to identify; they display clinical features of hypothyroidism but paradoxically near-normal thyroid function. TSH is normal, with higher fT3 and lower fT4, albeit often in the normal range (with fT3 in the upper half and fT4 in the lower half of the normal range) (Erbaş & Demir, 2020).

MRI studies in patients with RTH syndromes are limited, with some case reports of reduced cerebellar size and macrocephaly in those with RTHβ, but normal structural MRI findings reported in other cases (Moran and Chatterjee, 2015; Al Shidhani et al., 2021). In 2023, an imaging study was undertaken involving 21 individuals with RTHβ, compared to 21 controls (Rogge et al., 2023). They found changes in the corticospinal tract, increased cortical thickness in the superior parietal cortex and decreased grey matter volume in the bilateral inferior temporal cortex and thalamus. Further studies in RTH syndromes, using advanced MRI methodologies, may help inform our understanding of the action of thyroid hormones in the human brain.

Thyroid hormone transporter deficiency

MCT8 deficiency, also named Allan-Herndon-Dudley syndrome (ADHS, OMIM 300523), leads to tissue hypothyroidism within the brain, which impacts on the hypothalamic-pituitary-thyroid axis. The resulting lack of thyroid hormone sensed

by the pituitary prompts increased production of thyroid-stimulating hormone (TSH), leading to excess thyroid hormone production and a corresponding peripheral thyrotoxicosis. This deficiency is inherited in an X-linked manner, with the first human MCT8 genetic mutation identified in 2004. An international consortium of 47 centres confirmed 151 cases by 2019 (Groenweg et al., 2020). Only a handful had MRI imaging available, but cortical atrophy and delay in myelination observed.

The majority of MCT8 deficiency patients have profound to severe intellectual disability, but severity can vary depending on the specific genetic defect (Remerand et al., 2019). Speech and language issues are commonly observed. The reliance of humans on MCT8 has limited the development of animal models until the recent creation of a MCT8/OATP1C1 double knockout model.

OATP1C1, more prevalent in mice, serves as an alternative transporter in the blood-brain barrier, sharing a similar phenotype with MCT8 deficiency in murine models. The first human case of OATP1C1 deficiency was reported in 2018 (Strømme et al.), with brain specific hypothyroidism and gradual development of neurodegeneration and motor and intellectual disability. The transporter demonstrates specificity for T4 transport in astrocytes (Mayerl et al., 2012). One hypothesis posits that reduced T4 conversion to T3 in astrocytes may lead to decreased T3 availability for neighbouring neurons (Morte & Bernal, 2014; Moran et al., 2022).

Table 1.1. Causes, biochemical findings and clinical signs of diseases of the thyroid in humans.

Condition	Causes	Biochemistry			Clinical signs
		TSH	fT4	fT3	
Euthyroid	Normal	N	N	N	None
Non-thyroidal illness/ sick euthyroid	Acute illness (transient)	N or ↓	↓	↓	Variable, related to underlying illness
Primary hypothyroidism	Autoimmune, iatrogenic, congenital, iodine deficiency	↑	↓	↓	Fatigue, weight gain, cold intolerance, bradycardia, myxoedema
Subclinical hypothyroidism		↑	N	N	Often asymptomatic, may have mild symptoms of hypothyroidism
Secondary hypothyroidism	Hypopituitarism	N or ↓	↓	↓	Similar to primary hypothyroidism, but often with other pituitary hormone deficiencies
Hyperthyroidism	Autoimmune, transient thyroiditis, toxic adenoma/ multinodular goitre,	↓	↑	↑	Weight loss, heat intolerance, tachycardia, exophthalmos (Graves' disease)

Condition	Causes	Biochemistry			Clinical signs
		TSH	fT4	fT3	
Subclinical hyperthyroidism		↓	N	N	Often asymptomatic, may have mild symptoms of hyperthyroidism
Maternal hypo-thyroxinaemia	Iodine deficiency	N	Not appropriately increased compared to gestational normal ranges	N	Maternal fT4 should increase in early gestation. Often mild, and conflicting evidence may impact offspring neurodevelopment. Severe maternal iodine deficiency, can cause cretinism.
Thyroiditis	Autoimmune, viral, post-partum				Pain in thyroid region (subacute thyroiditis), possible symptoms of hypo- or hyperthyroidism
Goitre	Iodine deficiency, familial, multinodular, untreated hypothyroidism				Visible swelling in the neck, potential symptoms of hypo- or hyperthyroidism

Condition	Causes	Biochemistry			Clinical signs
		TSH	fT4	fT3	
Thyroid hormone resistance	RTHß	↑ or N	↑	↑ or N	Tachycardia, goitre, increased risk ADHD
	RTHα	N or slightly ↑	N or slightly ↓	N or slightly ↑	Growth and mental delay, constipation

Abbreviations: TSH, thyroid stimulating hormone; fT4, free thyroxine; fT3, free T3; RTHß,or α, resistance to thyroid hormone caused by mutation in thyroid hormone receptor ß or α; N, normal; ↑, increased; ↓, decreased; ADHD, attention deficit and hyperactivity disorder.

1.5 Pregnancy – a unique situation

Thyroid function changes dynamically in the mother when she becomes pregnant. The foetus is dependent on the mother's FT4 until the latter half of the first trimester when its own thyroid gland has developed and can start producing its own thyroid hormones (figure 1.4). However, the foetus cannot meet all of its demands and still depends to some degree on the supply of maternal thyroid hormone until birth. Figure 1.5 illustrates the interplay of pregnancy and thyroid hormones and DIO3 in the placenta, all coming together to provide adequate fT4 supply for the foetus. Premature infants are often hypothyroxinaemic, but thyroxine supplementation in those less than 28 weeks was not found to affect brain volume (Ng et al., 2013). The effect on IQ or behaviour in this population has not been widely studied, but there are suggestions that thyroxine supplementation has a positive impact on neurodevelopmental outcomes (Ng et al., 2020).

In the area of antenatal thyroid function, the term maternal hypothyroxinaemia describes the lack of an adequate rise in fT4 in response to the pregnancy. In hypothyroxinaemia, regardless of TSH level, fT4 concentration is low compared to values found in other pregnant women with adequate iodine intake at the same stage of pregnancy. The fT4 is often still in the so-called normal range for the general, non-pregnant population. However, thyroid hormone concentrations at the population level are a continuum, meaning that it can be difficult to define exactly what is meant by hypothyroxinaemia or suboptimal gestational thyroid function (SGTF). Studies have thus commonly used cut-offs, e.g. mothers with fT4 <5% centile in the Generation R study (Ghassabian et al., 2014) or in the CATS study, fT4 at or below the 2.5th percentile, and/or TSH >97.5th percentile, or both (Lazarus et al., 2012).

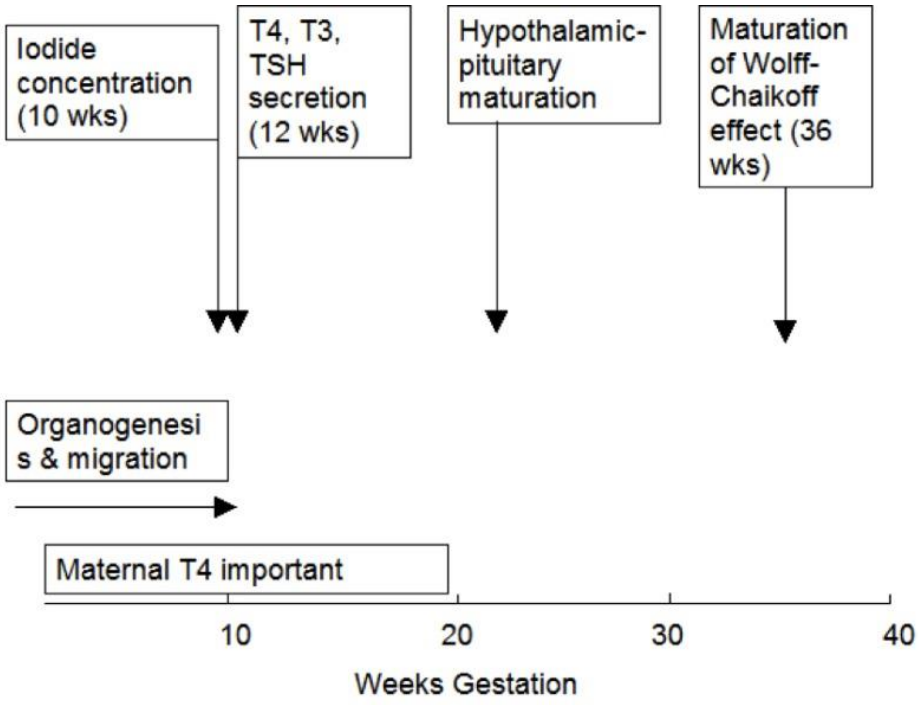


Figure 1.4. Approximate timing of thyroid gland maturation in the human foetus (Segni, 2017)

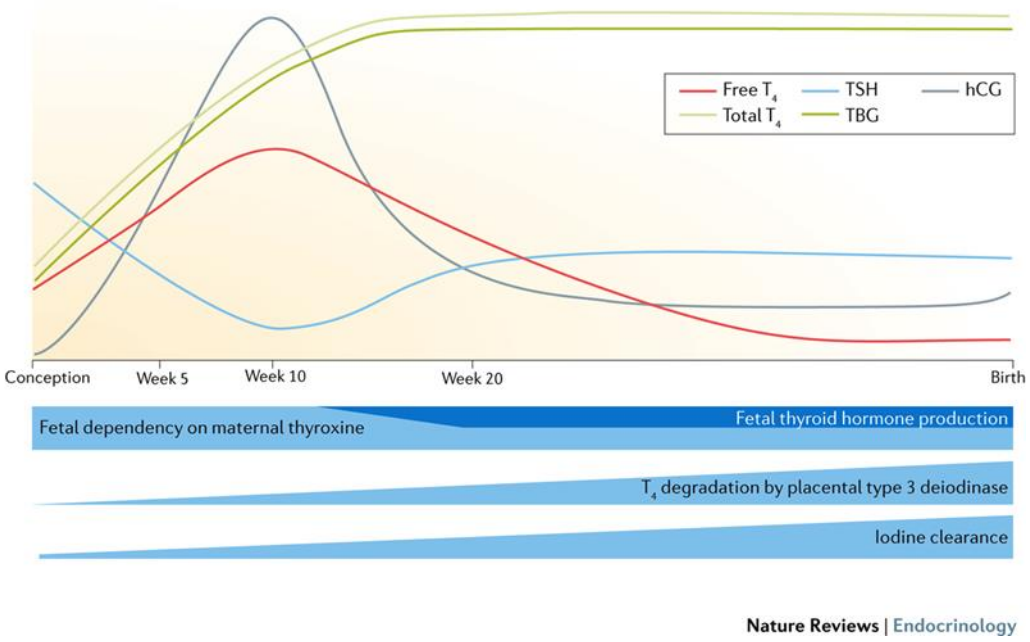


Figure 1.5. Changes in thyroid physiology during pregnancy (Figure 1 Korevaar et al, 2017). It includes the effects mediated via increased thyroidal stimulation by human chorionic gonadotropin (hCG), which leads to a net increase in free T4 concentration and a subsequent decrease in TSH concentration to counteract the increase in fT4 demand by the foetus via placental type 3 deiodinase expression.

1.6 Neurodevelopmental outcomes of thyroid disease in pregnancy and early life

If maternal T3 was able to cross the placenta, it would be at much higher concentrations than the foetus needed. Free T4 is inactive, and is allowed across the placenta since foetal tissues can control the rate and timing of conversion to active FT3 when needed. In the mammalian foetal brain, different anatomical areas will activate deiodinaises at specific timepoints in gestation according to species. This is thought to allow the foetus to ensure optimal spatio-temporal genomic actions of T3 (Galton et al., 2014). The exact timings in defined areas right at the point they are undergoing key neurodevelopment illustrates the importance of thyroid action on the developing brain.

Before the 1990s, it had been difficult to measure the small amounts of thyroid hormone in the foetus or access foetal compartments not in direct communication with the mother (Morreale de Escobar, 2000, 2004). The placenta was noted to have high level of DIO3 activity, which breaks down thyroid hormones and was thought to create a barrier to both thyroid hormones. Since both patients with neurological cretinism and untreated congenital hypothyroidism display severe learning disabilities, and before commercially available thyroid hormone assays were developed, these two conditions had sometimes been confused. The observation of normal neurodevelopmental outcomes in congenitally hypothyroid infants treated from birth with levothyroxine, led to a consensus for some time that the foetus did not receive a biologically meaningful amount of thyroid hormone during gestation. Therefore, the mother's contribution was thought to be less directly relevant, as long as the foetus received adequate iodine.

In the 1990s to mid-2000s, the group of de Escobar and Escobar del Rey published a series of manuscripts describing the distribution of thyroid hormones in the pregnant rat and foetus and the effect of iodine and hypothyroidism in pregnant rats on the offspring's neocorticogenesis (Bernal, 2018). The observation of similar concentrations of fT4 in the foetus as the mother, but not of fT3, encouraged a reconsideration of the importance of maternal thyroid function on foetal outcomes during pregnancy (Calvo et al., 1990; Morreale de Escobar, 2004).

Two of the iodothyronine deiodinaises, DIO2 and DIO3, have opposite actions and help modulate cellular exposure and response to thyroid hormones (Galton et al., 2014). DIO2 is present in the endoplasmic reticulum, and converts T4 to T3 for nuclear import, so modulating activity (Arrojo e Drigo et al., 2011). DIO3 is present on the plasma membrane, and inactivates thyroid hormones, so is thought to protect tissues from excess T3 (Arrojo e Drigo and Bianco, 2011). Morreale de Escobar (2004) provides a detailed overview of the evidence for the changing dynamics within the thyroid axis over the course of pregnancy. DIO2 switches on at different points in different places in the developing foetal brain. This converts T4 to active T3, whether maternal or foetal origin. In iodine-deficient mothers, the body will prioritise T3 production with available iodine; it is thought this is because T3 requires just 3 iodine atoms, not 4, and is the more biologically active. Therefore, TSH may stay in the normal range, but the rise in maternal T4 needed at the start of pregnancy cannot occur, and mothers can be hypothyroxinaemic. This is important for the foetus, as it is the maternal free T4 (fT4), not T3, that is vital in the first half of gestation, with DIO3 breaking down T3 and preventing maternal T3 crossing the placenta (Calvo et al., 1990; Bernal, 2015). The majority of circulating thyroid hormones are bound to thyroglobulin binding protein, forming a preformed reservoir of thyroid hormones that can be utilised rapidly when required. The term T4 represents the total amount of thyroxine in the bloodstream, whilst fT4 specifically measures the active, unbound form of the hormone that is directly available for metabolic processes.

1.7 Thyroid function and neurodevelopment

Neurodevelopment involves the biological formation and development of the nervous system, manifesting in motor, sensory, cognitive, and emotional functions. Various factors, including genetics, environment (nutrition, chemical exposure, social, emotional), play a role. Cognition refers to mental processes such as acquiring, processing, storing, and using information. Assessments of cognition and executive functions cover memory, attention, language, problem-solving, and perceptual-motor skills (Otterman et al., 2019).

Neurodevelopmental disorders encompass a broad range of clinical conditions resulting from disruptions in normal brain development. There are a wide range of clinical conditions resulting from a disruption of “normal” brain development, with the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM–5; American Psychiatric Association, 2013) defining them as conditions with onset during the developmental period, typically appearing before school entry and causing deficits affecting personal, social, academic, or occupational functioning. This term distinguishes disorders such as intellectual disability, communication and learning disorders, motor disorders, ADHD, and autism spectrum disorder (ASD) from later-onset neurocognitive and neuropsychiatric conditions. Recent more multi-dimensional approaches to measurement of symptoms demonstrate a range of severity rather than simple categories, with a blurred boundary with typical development. Diagnosis of a neurodevelopmental disorder therefore requires that both symptoms and impaired function are present (Thapar et al. 2017).

1.7.1 Preclinical models of role of thyroid hormones on nervous system development

Pre-clinical models allow the manipulation of thyroid hormone levels in a research environment, often using cell culture or animal models. Preclinical models are especially useful to investigate actions during early neurodevelopment.

Cellular/microscopic

The term neocorticogenesis describes the process of the primitive neural tube, with as yet undifferentiated cells, developing into the cortical plate, and neurones differentiating and migrating into an organised pattern. It is spatially timed and an early process in the development of the central nervous system, beginning before the fifth week of human gestation, when the foetus is still dependent on maternal thyroid function (Bernal, 2015; Segni, 2017). Lavado-Autric and colleagues (2003) were the first to publish evidence that early maternal thyroid dysfunction in completely iodine-deficient rats could alter foetal brain histogenesis and cytoarchitecture. They extended their observations to demonstrate that even a transient three-day period of early maternal hypothroxinaemia, without overt hypothyroidism, was sufficient to alter neurogenesis (Auso et al., 2004). The

timing was at a comparable time to the period of mid-gestation in the human foetus. In the rat pup brain, there were alterations in cytoarchitecture of the somatosensory cortex and hippocampus. Neuronal migration in the neocortex was also disturbed, similar to the previous study with more extreme, sustained thyroid disruption. The goitrogen used in the Auso et al. (2004) study is not known to be teratogenic.

Bernal's 2015 review of the effect of thyroid function on the brain provides a broad overview of mechanisms by which maternal thyroid dysfunction can affect foetal neurodevelopment. Thyroid hormones affect the transcription of the gene *Reln*, whose product Reeln is important for neuronal migration and architecture (Del Rio et al., 1997). T3 receptor mRNA is detected in the first trimester (Maia et al., 2005). Total brain T3 receptor concentration increases ten times during the active neuroblast proliferation and massive expansion in brain volume and development which occurs between the 10th and 16-18th week of gestation (Dobbing and Sands, 1970; Wejaphikul et al., 2019).

Neurogenesis

Thyroid hormones have a role in proliferation and differentiation of neural precursors in embryonic neurogenic areas in tadpoles during metamorphosis (Denver et al., 2009). However, in humans, most thyroid hormone effects appear to influence the later events of neural development and migration, and the terminal differentiation of neurones and glial cells. Thyroid hormone has been found to regulate the expression of the sonic hedgehog signalling pathway in the embryonic and adult Mammalian brain (Desouza et al., 2011). Thyroid hormones continue to influence neurogenesis in the adult, with hypothyroidism reducing, and levothyroxine replacement stimulating neurogenesis in subventricular and sub-granular zones (Berbel et al., 1994; Desouza et al., 2005; Kapoor et al., 2015; Lemkine et al., 2005).

Cell migration

Thyroid hormones have important actions in cell migration in the cerebral cortex, hippocampus, corpus callosum and cerebellum (Bernal, 2015; Cuevas et al., 2005). Effects on radial glia cells are thought to be a possible mechanism for this since they have structural and supportive functions and generate neurons and

neuronal precursors (Hatten et al., 1990; Krigstein et al., 2009; Martinez-Galan et al., 1997a, b, 2004). Deficiency in thyroid hormones during cortical development in rats causes less defined cortical layers and abnormal cell migration (Berbel et al., 1994, 2001). Thyroid hormones regulate the expression of the gene Reln in Cajal-Retzins cells, which produce Reelin. Reelin is an extracellular matrix protein and contributes to orderly neuronal migration (Del Rio et al., 1997; Garcia-Fernandez et al., 1997).

Myelination

In the foetus, differentiation of oligodendrocytes, which produce myelin, is affected by thyroid function. Deficiency in thyroid hormone during gestation delays differentiation and myelin gene expression. However, in rats completely deficient in T3 or T4, the oligodendrocytes in the foetus will eventually differentiate and produce myelin. However, the delay in myelination causes permanent defects in the adult rat, who display a reduced number of myelinated axons in response to prolonged neonatal hypothyroidism. The thickness of the myelin sheath appears unaffected (Adamo et al., 1990), in contrast to axonal maturation. It is postulated that the lower diameter of axons in hypothyroid animals could prevent axons reaching the critical size to become myelinated.

1.7.2 Clinical evidence of the impact of maternal thyroid dysfunction during gestation

In 1995, de Zegher et al. reported the crucial role of maternal T4 as a rescue mechanism for congenital hypothyroidism (CH) via the case report of a mother and child with foetomaternal Pit-1 deficiency causing TSH deficiency. The case emphasised the thyroid hormone's potent role in foetal brain maturation. It illustrated how placental transfer of maternal T4 serves as a rescue mechanism for infants with CH, safeguarding developmental potential. The index case had a *de novo* mutation, and *in utero*, was able to use her mother’s fT4. Levothyroxine was initiated during childhood.

During her first pregnancy, the mother’s levothyroxine was stopped during the second half of gestation, resulting in no fT4 supply for the foetus. The child, heterozygous for Pit-1, despite having a mother adequately replaced with thyroxine in the first half of gestation, and receiving treatment with thyroxine soon

after birth, still experienced severe neurodevelopmental damage due to the lack of T4 in the second half of gestation. Without foetal TSH stimulating endogenous T4 production, the developing foetus was completely reliant on maternal fT4. This case underscores the critical role of continuous T4 during gestation, including in the third trimester, in preventing irreversible damage in CH. The mother had adequate levothyroxine replacement during her second pregnancy, and her second child had normal neurodevelopment (Pine-Twaddell et al., 2013).

Less severe maternal thyroid dysfunction

Once severe maternal iodine deficiency and hypothyroidism had been mostly tackled, the research community moved on to consider if less severe forms of maternal thyroid function might also have a detrimental effect on offspring neurodevelopment. The non-cretin population in severely iodine-deficient areas, although born to mothers without severe hypothyroxinaemia, were found to have a statistically significant reduction in average IQ, together with increased rates of sensorineural hearing impairment compared to those living in similar, iodine-sufficient areas (Querido et al., 1975; Gosling et al., 1975).

Effect of maternal thyroid function on offspring IQ

Maternal thyroid function is not routinely checked during pregnancy in the absence of a prior history of thyroid disease. Haddow et al. (1999) raised the question of whether it should be part of antenatal assessment. They reported a significant reduction in IQ in children of mothers who had untreated or only partially treated hypothyroidism in pregnancy (Haddow et al.,1999). The first randomised controlled trial using an intervention then followed: the Controlled Antenatal Thyroid Screening (CATS) study by Lazarus et al. (2012).

The CATS trial was predominantly based in South Wales, with IQ and psychological assessment being conducted at age 3 years in the children of women found to have suboptimal gestational thyroid function (SGTF). When recruited at their 12 week antenatal review, participants were randomised to have thyroid function tested immediately or stored until they gave birth. Those randomised to have thyroid function tested during pregnancy were started on levothyroxine if were found to have SGTF (Lazarus et al., 2012). At 3 years of age, no difference in IQ between the offspring of the two groups, treated and

untreated SGTF, was found. However, no comparison was made with the normal GTF group, raising the question of whether the timing of starting levothyroxine could have been too late in gestation. The assessments were repeated in the CATS II study, where a proportion of the children were reassessed at age 9, this time also including the offspring of mothers who had had normal gestational thyroid function in the initial CATS study (Hales et al., 2018). CATS II confirmed the finding of the first CATS study, i.e. that there was no difference seen between the IQ of offspring of mothers who were treated or not for suboptimal gestational thyroid function, but also showed that these groups did not differ from normal GTF (Lazarus et al., 2012; Hales et al., 2018). In 2017, the American RCT study by Casey et al. (2017), which included an even larger number of pregnant mothers, also found no difference in IQ. The CATS studies are discussed in greater detail in chapter 3.

1.7.3 Suboptimal maternal thyroid function and associations with ADHD

Fetene et al.'s 2017 systematic review titled "maternal thyroid dysfunction during pregnancy and behavioural and psychiatric disorders of children" was the first systematic review on the subject. Thirteen articles met the inclusion criteria, with all based within Europe or the US. A variety of associations with gestational thyroid dysfunction was found, with the majority of studies (8/13) concerning attention deficit hyperactivity disorder (ADHD) risk. Autism spectrum disorders, externalising behaviour and epilepsy/seizure risk were other disorders studied.

In the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR), ADHD is defined as "a neurodevelopmental disorder defined by impairing levels of inattention, disorganization, and/or hyperactivity-impulsivity". The key word is "impairing", with symptoms that are deemed as excessive for age of developmental level (Koutsoklenis and Honkasilta, 2022). It appears there is "atypical brain structure in ADHD, implicating multiple neural systems" are involved (Friedman & Rapoport, 2015). Structures known to be associated with attention, working memory and cognitive control- all of which can be impacted in ADHD (Arnsten et al., 2012; Valera et al., 2007) and are of growing interest for impact of thyroid function on neurodevelopment.

Table 1.2 summarises the ADHD studies included in Fetene and colleagues' systematic review (2017), ranging from 1999 to 2015. The majority of the studies reported an association between low maternal thyroid function and ADHD traits. However, as hyperthyroidism is less common than hypothyroidism/ hypothyroxinaemia (Taylor et al., 2018), it was difficult to study the effects of gestational high FT4 on behavioural/psychiatric disorders in children.

The two earliest studies investigating potential associated between maternal thyroid function and ADHD were published in 1999 (Haddow et al.) and 2004 (Vermiglio et al.). The two studies had small study populations, but did report increased attentional deficits/ADHD prevalence in those children born to mothers with hypothyroidism or hypothyroxinaemia.

Several of the more recent studies are part of the Generation R Biobank research group (Kruithof et al., 2014). Modesto et al. (2015) found an increased risk of ADHD at age 8 in children whose mothers had hypothyroxinaemia in the first trimester. The study consisted of a large number of mother-child pairs and was able to adjust for a large number of potential cofounders, including sociodemographic cofounders and maternal antenatal mental health. In the same cohort, an increased ADHD risk was also associated with maternal autoimmune thyroiditis at approximately 13.5 weeks (Ghassabian et al., 2012) and high TSH (Ghassabian et al., 2011). Generation R is a prospective, population-based cohort study following a cohort from the city of Rotterdam from when they were a foetus to young adulthood. As such, it provides a large cohort who have been followed-up and had a range of different assessments over the course of their childhood and has resulted in several studies examining a number of outcomes related to maternal thyroid function during pregnancy.

A Danish register-based cohort study, consisting of just over 85000 mother-child pairs, found that a maternal diagnosis/treatment of hyperthyroidism soon after pregnancy was related to increased ADHD risk in the offspring (adjusted HR 1.23; 95% CI 1.05-1.44) (Andersen et al., 2014). This was not found in children of mothers treated for hyperthyroidism prior to pregnancy. One limitation is that the children were assessed for ADHD traits at a relatively young age of 3. In a French ADHD study, the mean age parents first identified symptoms of ADHD

was 4.03 years, with mean age of a formal diagnosis of ADHD at age 8.0 years of age (Caci et al., 2020). Andersen et al. (2014) also reported an association between maternal hypothyroidism and risk of autism spectrum disorder (ASD) in the offspring (adjusted HR 1.34; 95% CI 1.14-1.59). In contrast, the Generation R cohort study did not find an association between maternal TSH or fT4 in the normal range and ASD, but did report an increased risk of ASD in those children from the severe maternal hypothyroxinaemia group (fT4 <5% of the cohort) (Roman et al., 2013).

A more focused study from the Danish register-based cohort study was published in 2018 (Andersen et al.). A sub-set of the population cohort was selected, including mother:child pairs whose children were diagnosed with epilepsy, ASD, ADHD or another developmental disorder (2276), compared with controls (7624). Maternal thyroid function was measured median 9 weeks' gestation. Abnormal maternal thyroid function in early pregnancy was associated with a diagnosis of epilepsy, ASD, or ADHD in the child, but associations differed by subtypes of exposure and by child age and sex. The average age when an ADHD diagnosis was recorded was 8.4 years. A higher level than expected had abnormal maternal thyroid function results, 12.5% of the control group. This was significantly higher in the ASD group (17.9%; aHR = 1.5 [CI 1.1-2.1]), but not in the ADHD or epilepsy groups. Overt maternal hypo- or hyperthyroidism was associated with increased risk of ASD but not ADHD. Isolated maternal hypothyroxinaemia was associated with increased risk of an ADHD diagnosis (aHR = 2.3 [CI 1.2 – 4.3]) in girls but not boys. However, confidence intervals were wide due to the small number of cases in stratified analyses.

A Finnish study reported no overall association between high maternal TSH during pregnancy and ADHD traits in the offspring. A secondary analysis found a weak but statistically significant increased risk of ADHD traits raised maternal TSH in female but not male offspring (Päkkilä et al., 2014).

The CATS II study also included neurodevelopmental psychological assessments as well as IQ measurements. A proportion of the women randomised to treatment in CATS I were deemed to have been "over-treated", as they became hyperthyroid after starting levothyroxine and needed their dose reduced, and they

were included in the secondary analysis. In the CATS II study, there was no overall association between SGTF at 12 weeks' gestation and offspring neurodevelopment questionnaire scores at age 9, which included symptoms associated with ADHD. However, children of the 'over-treated' mothers displayed significantly more ADHD traits than those with normal gestational thyroid function (Hales et al., 2020).

The majority of research suggesting an association between thyroid function and increased risk of ASD or ADHD traits' or diagnosis has focused on maternal thyroid function in pregnancy. A small number of studies have reported increased risk of ADHD or ASD traits in children born with congenital hypothyroidism (Rovet et al., 1999; Álvarez et al., 2010), but others found no association (Kooistra et al., 2001 & 2004). A prospective study following up children diagnosed with congenital hypothyroidism from birth found those who experienced under or over-replacement of thyroxine during the first 6 months postnatally had increased risk at age 10 years of ADHD if one or more episodes of over-replacement or ASD traits if one or more episodes of undertreatment as infants (Bongers-Schokking et al., 2018).

Table 1.2 Studies examining the risk of ADHD in children exposed to maternal thyroid dysfunction during gestation. Adapted and expanded from Fetene et al. (2017).

Author	**Title**	**Main findings**
Haddow USA 1999	Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child	Reported an association between maternal hypothyroidism & offspring's attention at 7-9 years old. Did not adjust for cofounders. Included 62 mother-child pairs with gestational overt hypothyroidism based on TSH levels, with 124 matched control pairs. The same paper included data on IQ, as discussed in main text of this chapter.
Vermiglio Italy 2004	ADHD in the offspring of mothers exposed to mild-moderate iodine deficiency: a possible novel iodine deficiency disorder in developed countries	Prospective study. Reported association between moderately iodine deficient (ID) areas and hypothyroxinaemic mothers and ADHD prevalence. Very small study- 16 mother-child pairs in moderate iodine-deficient area and 11 control pairs in mildly iodine-deficient area. Observed a higher rate of hypothyroxinaemic mothers in the moderately ID area, but the ADHD prevalence seemed extremely high (68.3% in the moderate ID area) and may be a reflection of the very small numbers. Not all of the 16 mothers from the moderately ID area were hypothyroxinaemic. Authors did not adjust for cofounders.

Author	Title	Main findings
Chevrier USA 2011	Maternal thyroid function during the second half of pregnancy and child neurodevelopment at 6,12,24 & 60 months of age	In contrast to other studies, this study found a reduction in ADHD symptoms, as reported by mothers in 60-month-old children, in those exposed to higher TSH levels in gestation. However, the study authors found this was not supported by other measures of hyperactivity and/or inattention, and concluded no impact of maternal thyroid function in second half of pregnancy on offspring neurodevelopmental outcomes. Study size was small (287 mother: child pairs).
Ghassabian^ Netherlands 2011	Maternal thyroid function during pregnancy and behavioural problems in the offspring: Generation R study	Association between high TSH during pregnancy at 13.3 weeks and ADHD.
Ghassabian^ Netherlands 2012	Maternal thyroid autoimmunity during pregnancy and risk of ADHD problems in children: Generation R	Association between maternal autoimmune thyroiditis at 13.5 weeks gestation and increased ADHD risk.
Päkkilä Finland 2014	The impact of gestational thyroid hormone concentrations on	No overall association, but increased risk of ADHD issues in female offspring of mothers with higher TSH. Prospective, population-based Northern

Author	Title	Main findings
	ADHD symptoms of the child	Finland Birth Cohort 1986. ADHD assessed by teachers completing questionnaires for 5131 children at age 8 years old.
Andersen Denmark 2014	Attention deficit hyperactivity disorder and autism spectrum disorder in children born to mothers with thyroid dysfunction: a Danish nationwide cohort study	Maternal hyperthyroidism only diagnosed after birth was associated with increased ADHD in the offspring at age 3 years. Not found in mothers already diagnosed and treated for thyroid disease before pregnancy. However, 3 years old may be too early an age to judge ADHD. A large register based, retrospective cohort, using ICD-10 hospital codes. >85000 M-C pairs. Did not record gestational age when thyroid function was measured.
Modesto^ Netherlands 2015	Maternal mild thyroid hormone insufficiency in early pregnancy and ADHD symptoms in children: Generation R	Hypothyroxinaemic mothers at mean gestational age 13.6 weeks had progeny with increased ADHD rates at 8 years old. Adjusted for a large number of cofounders, including sociodemographic factors and maternal antenatal mental health.
Andersen Denmark 2018	Maternal Thyroid Function in Early Pregnancy and Child Neurodevelopmental Disorders: A Danish	Maternal hypothyroxinaemia associated with ADHD diagnosis in girls but not boys.

Author	Title	Main findings
	Nationwide Case-Cohort Study	

^ Studies resulting from Generation R Biobank study

ASD: autism spectrum disorder; ADHD: attention deficit hyperactivity disorder.

Neuroimaging studies

The precise mechanism/ locations of alterations in the developing brain resulting from abnormal maternal or early childhood thyroid function remains uncertain. MRI neuroimaging offers an opportunity to investigate the underlying issues in brain development that underpin the clinical manifestation in a safe and non-invasive approach, with greater detail and versality than computed tomography (CT) imaging. MRI has been used extensively for investigating ADHD in children, but so far, few neuroimaging studies investigating effect of maternal thyroid function have been published. However, MRI studies can be resource heavy and time-consuming, requiring greater logistical considerations for the study design than simply measuring serum levels or evaluating for presence of a goitre.

The number of studies of thyroid function effects on neurodevelopment are much smaller than the field of ADHD research, but some studies investigating gestational thyroid function suggest potential associations with ADHD and other cognitive/executive function impacts. As MRI scanners have improved and a greater body of imaging data published regarding brain development, a small number of studies have been undertaken investigating the effects of thyroid hormones on the brain. However, modern MRI techniques have only emerged after the prevention of most cases of cretinism and the early detection and treatment for CH. MRI studies in thyroid dysfunction are thus predominantly in suboptimal/subclinical disease. To ensure that all relevant studies were found and reviewed, I undertook a systematic review (described in chapter 3) with a view to informing the analysis protocol I adopted in chapters 4.

1.8 Hypothesis and Aims

Based on previous studies, including the CATS II study, which reported associations of maternal thyroid dysfunction with increased risk of ADHD traits, I hypothesised that abnormal thyroid hormone bioavailability during neonatal development could alter brain maturation with potential impact on brain morphology. The follow-up to the CATS II study has been the CATS III study, which focused on undertaking MRI neuroimaging of children involved in the CATS II study.

To test this hypothesis I sought to:

1. Undertake a systematic review of the current MRI neuroimaging literature concerning exposure to abnormal thyroid function during childhood, including gestation.
2. Use MRI in the CATS III study to establish if altered maternal thyroid availability during gestation is associated with altered brain morphology.

Chapter 2

Chapter 2. Principles of magnetic resonance imaging

2.1 Introduction

Magnetic resonance imaging (MRI) is a non-invasive medical imaging technique used to visualize detailed internal structures of the body, especially soft tissues, with high clarity. The aim of this chapter is outline the essential principles of MRI and the main types of MRI techniques currently employed in neuroimaging research.

Unlike X-rays and computed tomography (CT) scans, which use ionizing radiation, MRI employs powerful magnetic fields and radiofrequency waves to produce detailed images. MRI is of particular use for imaging the brain, providing contrast between different soft tissues. In addition to its use in diagnosing and monitoring a wide range of medical conditions, it has a significant place in neurology research. Advanced MRI modalities, such as functional MRI (fMRI), diffusion tensor imaging (DTI), and magnetic resonance spectroscopy (MRS), offer insights into brain activity, neural pathways, and biochemical changes, important for both research and increasingly clinical uses.

2.1.1 The development of MRI

Isidor Isaac Rabi was awarded the 1944 Nobel prize in physics for his “resonance method for recording the magnetic properties of atomic nuclei”. He developed the atomic and molecular beam magnetic resonance method of observing atomic spectra, measuring their magnetic properties. Purcell and Block won the 1952 Nobel prize in physics for developing methods for studying the magnetic resonance properties in solids and liquids, not just individual atoms or molecules. In the 1970s, these principles were developed into the clinical imaging technique of MRI. The first live human subject was imaged in 1977, with MRIs becoming commercially available for clinical use in the 1980s. The 2003 Nobel prize in physiology or medicine was awarded to Paul Lauterbur, who developed gradient fields to allow allocating signal spatiality and Peter Mansfield, who developed the mathematical methods needed to allow radiofrequency (RF) signals to be converted into image signals.

2.1.2 Key principles of MRI

Structural MRI is the cornerstone of diagnostic neuroimaging used in the hospital setting. In recent years, an increasing variety of MRI techniques, first developed for research, are now being incorporated into medical use, e.g. planning neurosurgical approaches using functional MRI (fMRI) or diffusion tensor imaging (DTI).

The simplistic explanation of how magnetic resonance images are acquired is as follows: the patient is placed within the bore of a large magnet. The magnetic field temporarily realigns hydrogen atoms in the body. Radiofrequency waves then cause these aligned atoms to produce faint signals, which are detected by the scanner and used to construct detailed images of the body's internal anatomy.

Protons and neutrons are the building blocks of atomic nuclei. By applying a strong external magnetic field (B0), the nucelus of the atom attempts to align to be either parallel with or perpendicular to the external field. If parallel to the magnetic field, the nucleus is deemed to be in a high energy state, and if perpendicular, in a low energy state. Nuclei with spin properties can be excited within the static magnetic field (B0) by application of a second radiofrequency magnetic field (B1), When B1 is applied perpendicular to B0, the nucleus will absorb this energy, and will transition between the two energy states. The energy being absorbed and then released creates a voltage that can be detected by the coil of wire within the MRI scanner.

In MRI, the fundamental measurement is the magnetism inherent in certain nuclear isotopes, particularly the hydrogen atom (1H), as it is present in water within the tissues of the body. This magnetism is referred to as spin. A strong, static magnetic field (B0) is used to align the spins of the nuclei, creating a magnetic density within the patient's body. When the magnetic field is aligned parallel with the external field, it causes a low-energy state. A high energy state is caused when B0 is aligned perpendicular to the external field. A second, rotating magnetic field (B1 or RF pulse) is applied for a short duration, which causes the nuclei to absorb this energy and transition between the two energy states. The second pulse rotates the nucleus, making it spin away from the direction of B0, a process called excitation. After the RF pulse is turned off, the spins rotate about

B0, termed precession. As long as the spins are aligned, i.e. in phase coherence, they create rapidly oscillating magnetic fields, detected by a receiver coil. However, this coherence decays rapidly over time, with a duration roughly given by the T2 time constant. During this decay in alignment, magnetisation reforms along the direction of B0. Small variations in the magnetic field experienced by the nuclei can lead to significant effects on the MRI image. These variations can be caused by factors like magnet design, tissue properties (susceptibility), and the nuclear environment within molecules.

The energy and magnetic energy is created by supercooling coils of wire within a MRI scanner using liquid helium. By cooling the coils close to absolute zero (0K), it allows massive electrical currents to be conducted, creating cryogenic superconducting magnets. With the advent of MR field gradients, three sets of coils can apply gradients in different directions, enabling different MR signals to be detected at different positions in space, and allow images to be reconstructed in 3 dimensions. Higher field strength systems result in improved signal to noise ratios, leading to higher resolution images and improved quantifications. The unit of measurement of MRI field strength is a Tesla (T). The majority of clinical MRI scanners use 1.5T, but the newer generation are 3T scanners.

2.2 Common MRI Techniques in Neuroimaging

2.2.1 Structural MRI

T1- and T2-weighted MRI is the cornerstone of clinical diagnosis and monitoring of disease. In structural MRI there are two different types of relaxation, i.e. the process by which a nuclear “spin” returns to thermal equilibrium after absorbing radiofrequency energy. T1-weighted MRI images are characterized by short repetition time (TR) and short echo time (TE), resulting in images where tissues with shorter T1 relaxation times appear bright, while those with longer T1 relaxation times appear dark. This means that tissues like white matter appear bright, while fluids and cerebrospinal fluid (CSF) appear dark. T1-weighted images are particularly good at highlighting the contrast between grey and white matter in the brain.

T2-weighted MRI images, in contrast to T1-weighted, have longer TR and TE, resulting in images where tissues with longer T2 relaxation times appear bright, while those with shorter T2 relaxation times appear dark. This makes fluids and CSF appear bright, while white matter appears darker than in T1-weighted images. T2-weighted images are sensitive to differences in water content and are useful for detecting abnormalities such as oedema, inflammation, and lesions, as these conditions often involve changes in water content within tissues.

Although T1-weighted MRI can be good to differentiate between white and grey matter in the brain, many of the white fibre tracts cross each other, making it hard to establish connections between different parts of the brain. Even in anatomical and histological studies, it is very hard to establish the specific white matter tracts.

2.2.2 Diffusion MRI

Diffusion MRI is concerned with functional connectivity of different structures, particularly white matter tracts, using the movement of water molecules. Diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) are related but distinct MRI techniques. DWI measures the random motion of water molecules within tissues, providing qualitative information about tissue microstructure and aiding in the detection of conditions such as acute stroke. In contrast, DTI extends DWI by quantifying the directionality of water diffusion using multiple diffusion-weighted images, allowing for the visualization and quantification of white matter fibre tracts in the brain. DTI metrics offer quantitative measures of tissue integrity and can be used to study various neurological conditions affecting white matter. Figure 2.1 illustrates neurones and the distribution of water and macro- and micromolecular content that MRI can use to investigate.

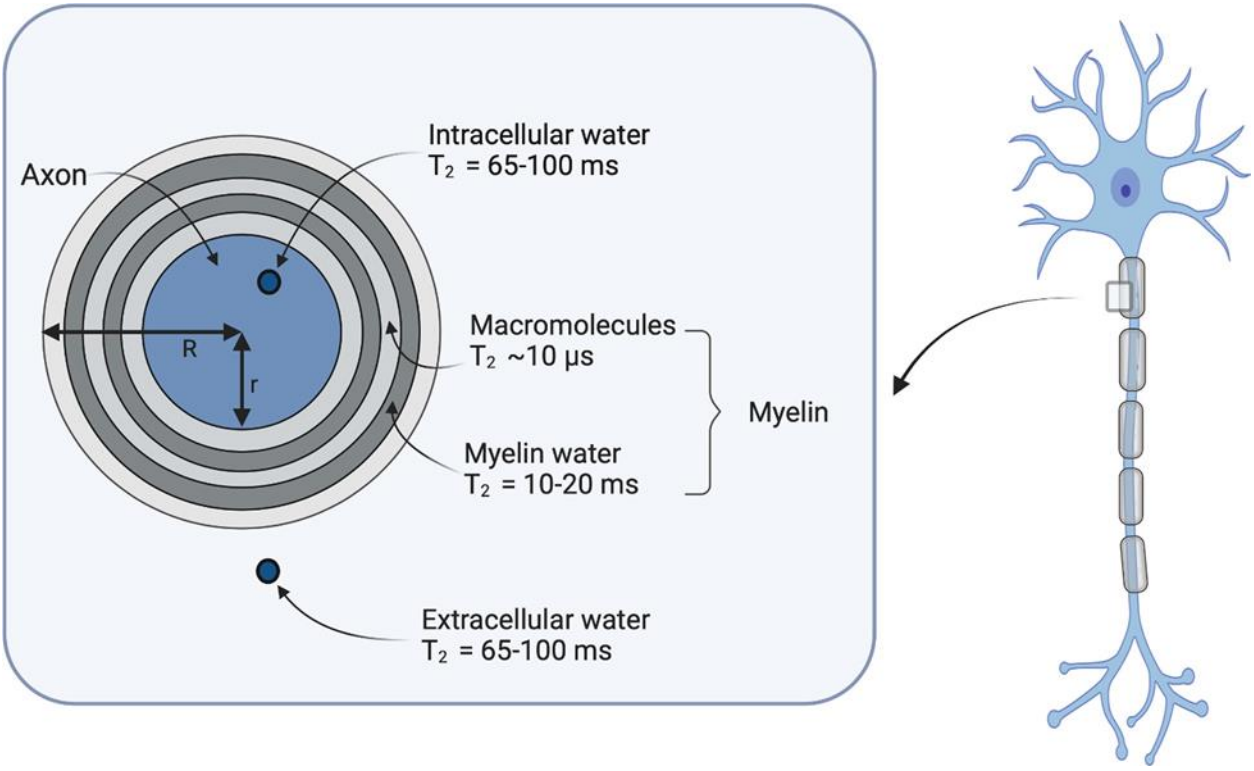
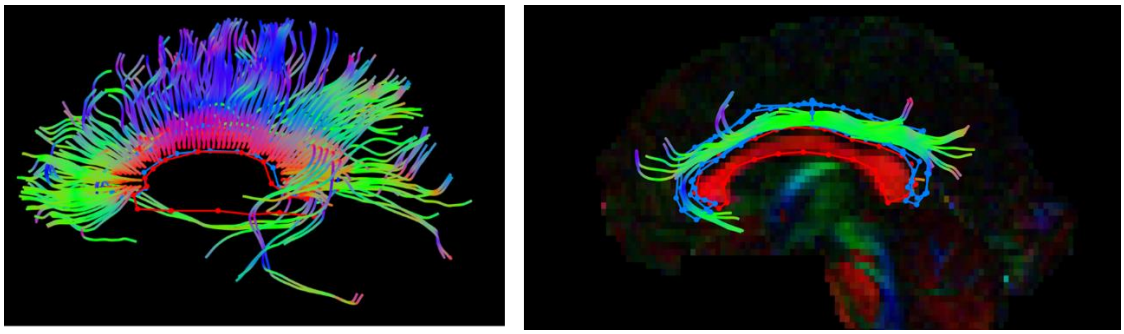


Figure 2.1 Representation of characteristics relevant for MRI in the central nervous system, focus on intra-axonal water and myelin. Myelin is wrapped around the axon. Within the axon is intracellular water, and outside the myelin layer is extracellular water. The myelin sheath consists of myelin water and lipid bilayers that contain macromolecules. Figure from van der Weijden et al. (2023), based on Fig. 1 from Campbell et al. (2018).

Fractional anisotropy and radial, axial and mean diffusivities are common metrics used in DTI. Diffusivities are scalar indices that describe the diffusion of water in specific voxels related to the structure of white matter tracts (Winklewski et al., 2018). Voxels can be thought of as three dimensional version of pixels and are the smallest volumetric elements in an MRI image. In neuroimaging, fractional anisotropy (FA) quantifies the overall magnitude of water's directional movement along axonal fibres, while mean diffusivity measures the average diffusion in all directions. Other commonly reported metrics include axial diffusivity (AD), which represents the average diffusion of water molecules parallel to the tract within the voxel of interest, and radial diffusivity (RD), which measures the magnitude of water diffusion perpendicular to the tract.

In Diffusion Tensor Imaging (DTI) MRI studies of white matter, streamlines depict the main directions of water diffusion, helping visualise the pathways of white matter tracts. They enable tractography, which maps neural connections and helps deduce connectivity patterns between different brain regions. Streamlines also indicate the structural integrity of white matter, with disruptions potentially pointing to damage or abnormalities. Quantitative analysis of streamline characteristics aids in evaluating microstructural properties, offering valuable insights into both normal brain function and various neurological conditions.

Tractography is a method to visualise white matter tracts and uses the scalar indices in individual voxels to project 3D trajectories of fibre pathways and connection between different brain systems *in vivo*.(Soares et al., 2013). Fibres and tracts will be crossing each other, making it difficult to accurately reconstruct a fibre tract. A range of different approaches and techniques and software can be used to try to generate the best estimate, but it will still be an estimate, and results have to be interpreted with a high degree of caution.



A. **B.**

Figure 2.2. Tractography of corpus callosum using ExploreDTI; A) Tractography of cingulum (green) and corpus callosum (red) including connections to other parts of the brain and B) using hand drawn region of interest (ROI) to isolate the tracts of interest. Images from a CATS III study participant.

2.2.3 Functional MRI (fMRI)

For a hundred years prior to fMRI being developed, blood flow was known to increase in areas of the brain undergoing activation (Roy & Sherrington, 1890; Kety & Schmidt, 1948). Functional MRI was developed in the 1990s, based on the magnetic properties of paramagnetic deoxyhaemoglobin; blood oxygenation

level-dependent (BOLD) contrast follows blood oxygen changes (Ogawa et al., 1990).

fMRI offers high spatial resolution, allowing localisation of brain activity to specific regions or substructures. fMRI also provides whole-brain coverage, offering a comprehensive view of brain function. Although its temporal resolution is limited, fMRI still allows for the observation of changes in brain activity over time, typically seconds. This versatility enables the study of various cognitive functions, such as language, memory, and emotion. Clinically it is used in helping to plan neurosurgical approaches.

2.2.4 Magnetic resonance spectroscopy (MRS)

Magnetic resonance spectroscopy imaging measures chemical shift of certain atomic nuclei. As well as 1H, 31P, 13C, 23Na and 19F also have “spin” properties, allowing them to be measured during MRS investigation. Results are often reported as metabolic ratios to a stable metabolite naturally occurring in the tissue, such as creatine (Cr). It is difficult to provide absolute quantifications of metabolites, due to a variety of factors affecting the measurements, including T1 and T2 signals (Grover et al., 2015).

Cerebral proton spectroscopy – visible metabolites.

• N-acetyl aspartate:

In MRS, N-acetyl aspartate (NACC) is used as a marker of neuronal dysfunction and neuronal loss (Vion-Dury et al, 1994), although its exact physiological function is unknown (Birken et al., 1989; Grover et al., 2015).

• Choline:

Phosphocholine molecules are released during membrane breakdown, therefore can be a marker of membrane activity. They are also involved in phospholipid metabolism and osmotic regulation in glial cells. Elevated choline resonance occurs in inflammatory process and is thought to be a marker of myelin breakdown (Davie et al., 1993).

• Creatine

The total peak from creatine and phosphocreatine is thought to be fairly constant in both the healthy and diseased brain, so is often used as an internal reference interval (Gadian, 1995)

2.2.5 Quantitative Magnetisation Transfer (qMT)

Quantitative Magnetisation Transfer (qMT) imaging is a specialised MRI technique used in neuroimaging research. It focuses on measuring the exchange of magnetisation between protons bound to free water molecules and protons bound to macromolecules (such as proteins and lipids) in biological tissues (van der Weijden et al., 2023), see figure 2.1 above.

In qMT, two MRI scans are acquired, allowing the signal intensities in the two images to be compared. Typically, the scans are a standard proton density weighted (PDw) image and a magnetisation transfer weighted (MTw) image. The MTw image is acquired with an additional radiofrequency (RF) pulse that selectively saturates the macromolecular protons. By comparing the signal intensities in these two images, researchers can derive quantitative metrics related to the macromolecular content and their interaction with free water (Campbell et al., 2018). It can offer more information about myelin content, axonal density, and tissue integrity than conventional MRI.

2.3 Pre-processing and analysis steps in neuroimaging

Pre-processing prepares the data for analysis. The aim is ensure the different images acquired during the scan are correctly registered together and aligned with a brain atlas to help analysis of certain structures, minimise noise and correct for potential artifacts. The brain extraction tool (BET) removes non-brain structures, e.g. skull and scalp. These structures are not of interest to analysis and is also part of the process of anonymising participants. Removing the skull, otherwise termed skull stripping, also removes the ability to reconstruct the participant’s face, which is especially important in a time of increasingly open data and collaboration.

Participants can move slightly during scanning, causing motion artifacts. Motion correction algorithms help to align images to reduce the impact of motion. Registration of images involves aligning different types of imaging sequences

taken at different times during the MRI scan of a participant. Registration corrects for differences in positioning, scaling, and orientation, ensuring accurate comparison and analysis. This allows comparison of images across subjects or across different modalities. There are several brain atlases that can be used to help allow automatic segmentation of brain structures for analysis, such as that used by the FreeSurfer software (Fischl, 2012).

Pre-processing prepares the data for analysis by ensuring that the different images acquired during the scan are correctly registered and aligned with a brain atlas. This step is crucial for analysing specific structures, minimising noise, and correcting for potential artifacts. The Brain Extraction Tool (BET) removes non-brain structures such as the skull and scalp.

During scanning, even if participants stayed absolutely still, they will naturally move slightly, e.g. breathing. This will cause motion artifacts, but often there will be other types of participant movement too, as the scan time is at least several minutes or up to an hour or more depending on technique being used and aim of the scan. Motion correction algorithms align images to mitigate the impact of this movement. Registration of images involves aligning different imaging sequences taken at various times during the MRI scan, correcting for differences in positioning, scaling, and orientation. This alignment ensures accurate comparison and analysis across subjects or different modalities.

Several brain atlases, such as those used by the FreeSurfer software, facilitate automatic segmentation of brain structures for analysis. These tools enhance the accuracy and efficiency of pre-processing, allowing for more precise and reliable analysis of neuroimaging data. An example of the automatic segmentation is below in figure 2.3.

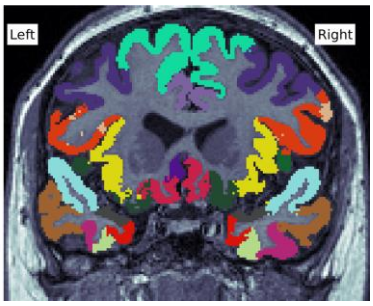


Figure 2.3. Example of automatic segmentation of cortical grey matter using Freesurfer. In image, the brain extraction tool has not yet been applied. Image from a CATS III study participant.

2.4 Applications and Challenges

Applications

The clinical applications of MRI range from diagnosing and monitoring neurological disorders such as multiple sclerosis, Alzheimer's disease and brain tumours to researching brain structure and function using functional MRI (fMRI), diffusion tensor imaging (DTI), and perfusion imaging.

Challenges and Limitations

MRI allows detailed analysis of the brain in human subjects, without invasive measures. It is an important clinical and research tool. However, there are challenges in data quality, methodology used, how to interpret results, high cost and tolerance to participants. Tolerance to the subject being scanned is important, as the bore of the scanner is narrow and can feel claustrophobic, coupled with having to lie very still and the movement of the magnets inside the scanner can be very loud.

Achieving high spatial and temporal resolution simultaneously poses difficulties, since higher resolution improves image quality but extends scan time and heightens susceptibility to motion artifacts. Image artifacts such as motion, magnetic susceptibility, and radiofrequency interference can distort images, complicating interpretation. Participants with claustrophobia can struggle or be unable to tolerate the scan. The noise can also be distressing to some people,

despite ear plugs. Distraction measures have been developed, such as mirrors or television screens, which are especially helpful when scanning children.

Data analysis can be complex, as MRI data requires specialised software and expertise for each stage of pre-processing, registration, segmentation, and statistical analysis. The cost and accessibility of MRI systems can present barriers, both for clinical and research use. MRI scanners are expensive both to procure and maintain, limiting availability in some regions and populations due to high scan costs. Additionally, interpretation challenges arise, as methodology of the scan, processing and analysis can affect the results. Furthermore, there is an ongoing debate about how brain structure and function interact, and how they influence one another. The key to building up a more robust idea of what different MRI technique results mean is to combine with other measures of brain function, such as executive functioning or motor tasks, other imaging techniques and/or histology from post-mortem or animal studies.

2.5 Conclusion

Overall, MRI plays an important role in both clinical practice and neuroscience research. MRI can provide non-invasive, detailed images of the brain's structure, function, and metabolism. It is a versatile tool, with the ability to capture multiple aspects of brain anatomy and physiology, both for research and clinical needs.

MRI is an important tool in neuroimaging, providing increasingly high spatial resolution and soft tissue contrast without ionising radiation. Challenges exist, including the need to balance spatial and temporal resolution, addressing image artifacts, data analysis, and managing costs and accessibility. Future developments aim to enhance MRI capabilities through technologies such as ultra-high field MRI, multi-modal imaging, machine learning and artificial intelligence (AI), real-time imaging, and personalised medicine, with the aim to lead to improved understanding of brain development and function and diagnostic accuracy and treatment outcomes.

Chapter 3

Chapter 3. Systematic review of brain MRI findings in children exposed to thyroid dysfunction during gestation or childhood

3.1 Introduction

Severe thyroid hormone deficiency during early gestation causes permanent neurological disability. Although this is well-documented, neuroimaging was not available at the time of the majority of studies undertaken, and the areas of the brain that are affected are unclear. In the 21st century, focus has moved onto the milder forms of maternal thyroid hormone dysfunction. However, the impact of mild suboptimal gestational thyroid function (SGTF) on IQ is debateable. As discussed in chapter 1, epidemiological studies and our observations in the CATS II study suggest that there may be an increased risk of offspring having increased risk of ADHD in those with CH or born to mothers with overt or subclinical thyroid disease. This implies there may be associated adverse neurodevelopmental sequelae.

The literature search for the introduction chapter of this thesis identified two main research groups focusing on maternal thyroid function (MTF) and congenital hypothyroidism (CH). While cretinism has been almost eradicated, some case reports persist in lower-income countries, suggesting the possibility of small studies from less well-known research groups. Childhood is a critical period for continuing neurodevelopment, and it remains unclear which brain regions might be most affected by thyroid issues. Therefore, a search was conducted to find any studies with neuroimaging results involving individuals exposed to thyroid dysfunction during the antenatal or childhood periods. The goal was to identify specific brain regions for further study in the CATS III analysis. Hyperthyroidism presents additional challenges for research, due to it being less common than hypothyroidism, and my initial literature search did not identify any neuroimaging studies related to it.

The objective of this systematic review is to identify previous neuroimaging studies looking at associations between brain development and exposure to abnormal thyroid function during gestation and childhood. The MRI data will hopefully give further insight into the areas of the developing brain most sensitive to thyroid hormones. Identifying these areas will help to guide future imaging studies and build on the histological findings from animal models. Of interest are any type of exposure to abnormal thyroid function throughout gestation and childhood. The desired outcome is to identify which brain areas in human children appear most affected, and

potentially identify key timings in development, both to help identify mechanisms of TH action on the developing brain, but also to identify whether any potential interventions could be implemented.

3.2 Methods

Methodology broadly follows the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) criteria (Moher et al., 2009), see appendix 7.2 for the updated 2020 PRISMA checklist. It does not meet the PRISMA standards in that there only one reviewer, not two independent reviewers.

3.2.1 Search criteria

The search for studies was originally undertaken on 24th July 2019, with a focused search undertaken in March 2021 in case of further relevant publications. The MeSH terms (Medical Subject Headings) of “magnetic resonance imaging”, “neuroimaging”, “diffusion magnetic resonance imaging”, “thyroid hormones”, “thyroid gland” and “thyroid diseases” were used to search Medline, with free text keywords related to thyroid and/or brain, MRI, and maternal, gestation, foetus, congenital and childhood also employed. The searches were then repeated in EMBASE, SCOPUS and PsychInfo databases. Full search strategy is in Appendix 7.3, page 178.Reference lists of key journals in the field, citation lists of included studies and related reviews were hand searched.

The aim of this systematic review was to provide the first comprehensive review of a relatively small field of thyroid-related neuroimaging studies in young people. The number of relevant studies was expected to be small, leading to the decision to create broad search criteria that would likely result in a large number of titles to screen, but ensure capture of all related sub-areas of related research.

3.2.2 Study selection and eligibility criteria

One author independently reviewed manuscript titles, identifying abstracts for further scrutiny. If the abstracts appeared to meet eligibility criteria, the full text article was then read, and if still meeting eligibility criteria, were put forward for inclusion in the review.

Inclusion criteria

- Research subjects whose mothers' thyroid function was evaluated during the pregnancy, OR subjects who were exposed to abnormal thyroid hormone levels during childhood, i.e. before 18 years of age.
- MRI brain neuroimaging studies of above research subjects.
- Case-control, cohort or interventional studies published in peer-reviewed journals.
- Published in English.
- Full-text articles.

Exclusion criteria

- Non-MRI neuroimaging studies.
- Case reports.
- Hashimoto's encephalitis (autoimmune mediated encephalitis not thyroiditis).

The search criteria did not include criteria related to age in order not to miss any participants exposed to thyroid dysfunction during gestation undergoing brain MRI when they were adults.

Only full-text articles were included, but when a relevant conference abstract was found, every effort made to try to find any potential future publications related to it.

No case-reports were included in the results, as there are too many unknown confounders, but if relevant, were included in the discussion, as were relevant non-MRI imaging studies.

Search results from each database were combined in EndNoteWeb, which de-duplicated results. Any duplicated titles found during the title, abstract or full-text review stages were also removed. The titles were screened for relevance, and if relevant, their abstracts then reviewed. If the abstract appeared to meet the inclusion criteria, the next stage was review of the full text article. Data collection was started at the full text article stage, as described below, and any found to not meet the systematic review criteria were excluded, with reason recorded (Figure 3.1).

3.2.3 Data collection and analysis

Title, type and size of study, first author, year published, country in which the study was undertaken, type of thyroid dysfunction participants were exposed to, sex of participants and age at time of scan and thyroid testing were recorded. Type of MRI imaging (e.g. fMRI, structural), field strength and regions of interest studied were also captured. Data items were extracted from the published studies into a spreadsheet. A narrative analysis of the included studies was used. Meta-analysis was not conducted due to heterogeneity of the exposure and outcome variables.

3.2.4 Quality scoring and risk of bias

The methodological quality of each study was assessed for risk of bias using the Newcastle-Ottawa Scale (NOS) (Wells 2008). The NOS assesses the quality of design of non-randomised case-control and cohort studies. Scores are assigned for selection criteria, comparability and outcome (cohort) or exposure (case-control). A maximum score of 9 stars reflects highest quality.

3.3 Results

After duplicates were removed, 7602 titles and abstracts were screened, as presented in the PRISMA diagram (Figure 3.1). A total of 78 full texts were assessed, with the majority being excluded as they concerned participants affected by thyroid dysfunction beginning in adulthood. The PRISMA flow diagram illustrates full reasons for exclusion (Figure 3.1). An updated, focused search was undertaken in February 2021 to look for new studies published post the 2019 search. It used the keywords from the included studies from the initial systematic search. and found one new study that matched inclusion criteria (Cooper et al., 2019).

All included studies are summarised in table 3.2 at the end of this chapter, including details of citation, country, title, type of study, MRI modality and field strength, age when scanned, age at thyroid sampling/diagnosis, size of exposed: non-exposed, study quality and underlying thyroid condition.

Twenty-two studies were included, which naturally divided into three discrete sub-groups: maternal thyroid dysfunction (MTDF), congenital hypothyroidism (CH) and thyroid hypothyroxinaemia of prematurity (THOP). Each condition affects children at different stages of their early development: during gestation, the early neonatal period and premature birth respectively. The majority of the studies scanned children of school-age, between the ages of 7 to 16 years old. Two CH (Siragusa et al., 1997; Akinci et al., 2006) and two THOP (Ng et al., 2014; Hung et al., 2018) studies scanned neonates. Biological sex was fairly equally distributed between the studies, although some neonatal studies did not include sex data. Eighteen of the included studies were of a case-control design, including one randomised controlled study (Ng et al., 2014). The case-control studies were relatively small in size, with a median of 19 cases per study, ranging between a total size of 10 and 83 participants. An exception was one study of 652 participants, but it had a small number of cases compared to the number of controls (27:625) (Ghassabian et al, 2014). The two other Generation R studies were of a cohort design, with between 598 and 1981 children scanned (Korevaar et al, 2016; Jansen et al., 2019). Two of the THOP studies were of cohort design, with between 8 (Hung et al., 2018) and 52 (Scratch et al., 2014) useable scans each.

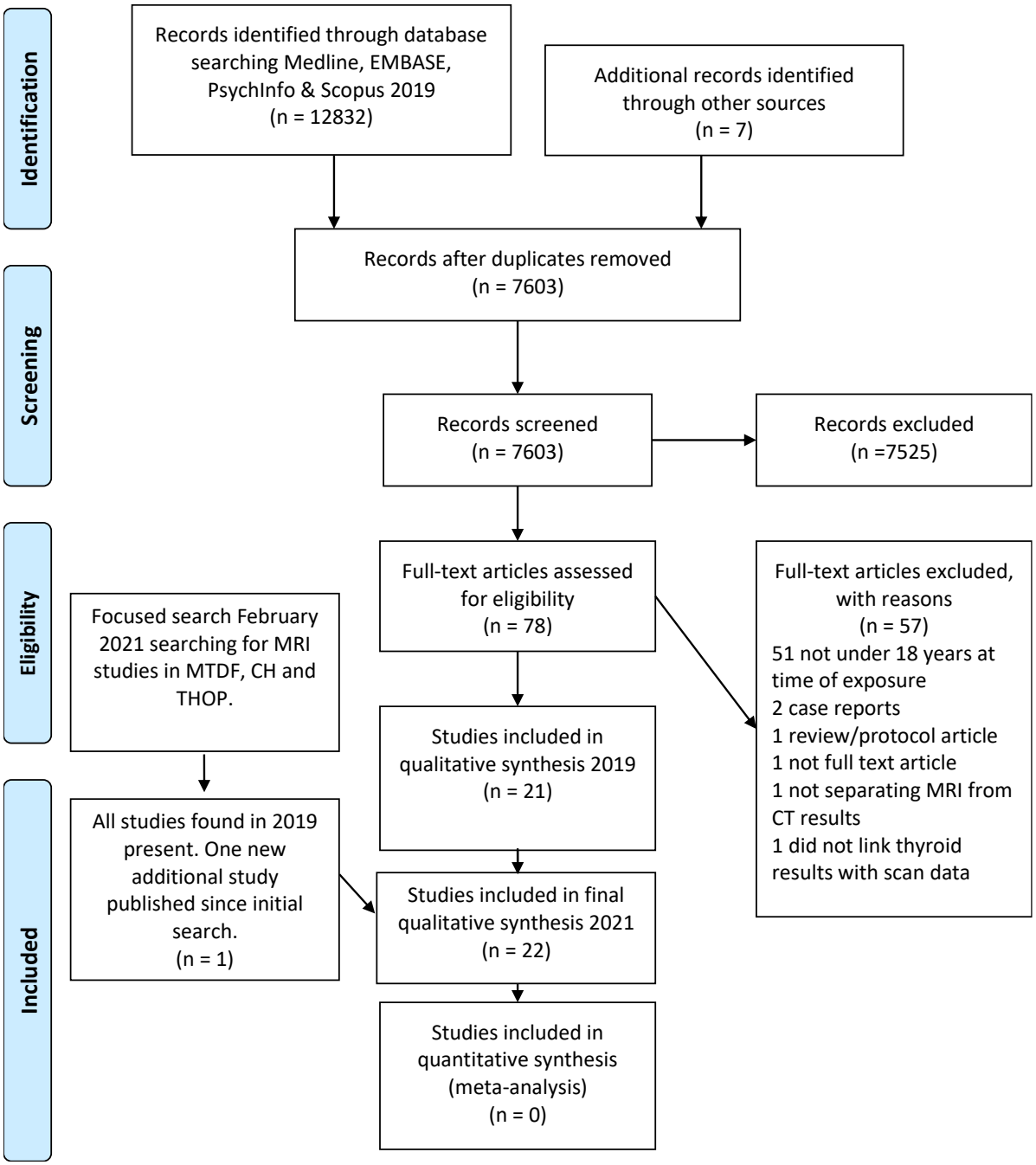


Figure 3.1 Adapted PRISMA (preferred reporting for systematic reviews and meta-analysis) diagram of July 2019 systematic search results, with addition of a focused updated search undertaken in 2021 to identify any further published studies meeting inclusion criteria. MTDF, maternal thyroid dysfunction; CH, congenital hypothyroidism; THOP, transient hypothyroxinaemia of prematurity.

3.3.1 Maternal Thyroid Dysfunction

MTDF is defined as any type of maternal thyroid dysfunction during pregnancy: hypothyroxinaemia, subclinical hypo- or hyperthyroidism or overt hypo- or hyperthyroidism. The effect of maternal thyroid function on offspring brain development was examined in seven studies between 2014 and 2019, from two research groups. All used structural MRI. One study included an additional group of children with congenital hypothyroidism (Willoughby, 2014a). Three studies originated from the Generation R cohort study in the Netherlands, using 3.0T structural MRI (Ghassabian et al., 2014; Korevaar et al., 2016; Jansen et al., 2019). The remaining four studies were from a Canadian group, using 1.5T MRI scan data (Willoughby, 2014 a & b; Samadi, 2015; Lischinsky, 2016). The majority of participants were scanned between the ages of 8 to 10 years (range 7 to 14 years).

The group from the Netherlands analysed maternal thyroid function and offspring MRI data acquired as part of the Generation R cohort study based in Rotterdam (White et al., 2018). The Generation R cohort produced the three largest studies included in this systematic review, ranging from 597 to 1981 mother-child pairs. The earliest Generation R study created a case-control design by defining exposed and non-exposed to hypothyroxinaemia (Ghassabian et al., 2014). The later two Generation R studies were conducted as population cohort studies, examining fT4 and TSH as continuous variables (Jansen et al., 2019; Korevaar et al., 2016).

The research group based in Toronto, Canada, examined the effects of maternal hypothyroidism (HYPO) on children's later brain development in four case-control studies (Willoughby et al., 2014a and 2014b; Samadi et al., 2015; Lischinsky et al., 2016). Compared to the Generation R studies, these studies were much smaller, with a total of between 42 to 64 participants. The MTDF was more marked than in Generation R, comparing children born to mothers with a diagnosis of overt hypothyroidism that was poorly controlled during pregnancy with children born to mothers without a known thyroid issue.

Two of the Generation R studies analysed the same archived MRI data of children who had undergone MRI at age 8 years. One study included only fT4 results (Ghassabian et al, 2014), the other undertaking a different analysis approach and

included the addition of TSH (Korevaar et al., 2016). Jansen et al. (2019) scanned 10-year-old children, some of whom are likely to have participated in the earlier neuroimaging studies, but this was not specified. The scans from the first MTDF neuroimaging studies published by the Canadian group (Willoughby et al., 2014a, Willoughby et al., 2014b) were re-used in later studies (Samadi et al., 2015; Lichinsky et al., 2016).

Quality assessment

The Canadian studies were small, but had demographically well-matched controls. However, issues with lack of thyroid results available for the control group and vague description of the recruitment of controls resulted in lower quality Newcastle-Ottawa scores of 5 out of 9 stars. The three generation R studies scored full marks on quality assessment.

Generation R Study

The Generation R study is a prospective cohort study based in Rotterdam, recruiting prenatally to follow participants from foetal life through to young adulthood. The aim of the study is to research environmental and genetic influences on children's growth, physical, cognitive and behavioural development, childhood diseases and healthcare for children and pregnant women. A total of 9778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study (>4000 children continue to be involved), with a subgroup invited for more detailed assessments (Jaddoe et al., 2006).

Ghassabian et al. (2014) compared children exposed to prenatal maternal hypothyroxinaemia, defined in their study as fT4 in the lowest 5% of the cohort's results (27/652), with the remaining participants defined as non-exposed controls. No relationship was found between maternal hypothyroxinaemia and offspring global brain volume, cortical thickness or brain surface area. Although the exposed group was small in comparison to the non-exposed, the participant characteristics did not differ significantly, apart from lower household income in the exposed group. Korevaar et al. (2016) re-analysed the same data, but incorporated TSH as well as fT4 in the main analysis. Instead of dividing the participants into groups based on a fT4 cut-off, the authors plotted data as a continuous variable. Korevaar et al. (2016)

reported an inverted U-shaped relationship between fT4 and grey matter volume, with grey matter volume being smaller at either end of the fT4 spectrum. No associations with maternal TSH values were found, nor between fT4 or TSH with total cerebral white matter volume.

Jansen et al. (2019) analysed a larger group of the Generation R cohort (1981 mother-child pairs) at the older age of 10 years. Part of the analysis approach was similar to Korevaar et al. (2016), with fT4 and TSH studied as continuous variables. Maternal fT4 (p=0.023), but not TSH concentrations, had an inverted U-shaped association with total brain volume. An inverted U-shaped relationship was again found between fT4 concentrations and grey matter volume, but statistical significance was lost after adjusting for total intracranial volume.

An additional analysis was undertaken in order to investigate the hypothesis of a time window where the foetal brain may be most vulnerable to maternal thyroid dysfunction (Jansen et al., 2019). When results were stratified by gestational age at time of maternal blood sampling (median 13.1 weeks, IQR 12.1-14.5 weeks), TSH but not fT4 was associated with significant differences in mean total grey matter volume (p=0.007) and mean cortical volume (p=0.022), both with an inverted U-shaped association (Figure 3.2). A similar trend was again seen with fT4, but this did not reach statistical significance. Results were most marked the earlier in gestation the blood sampling was done. Association was lost if maternal TFT was taken at from 14 weeks' gestation or later. Only one time point was available for each maternal thyroid function result, therefore no estimates can be made of potential trajectories over pregnancy.

The correlation of grey matter volume with first-trimester TSH rather than fT4 conflicted with the group's previous findings (Korevaar et al., 2016), hence the authors re-analysed the previous data gathered from the eight-year-old subjects. Undertaking sensitivity analyses and only including those with both TSH and fT4 results, Jansen et al. (2019) found that the earlier published data lost the statistically significant relationship with fT4, but did have a significant relationship with TSH, again in an inverted U-shaped pattern.

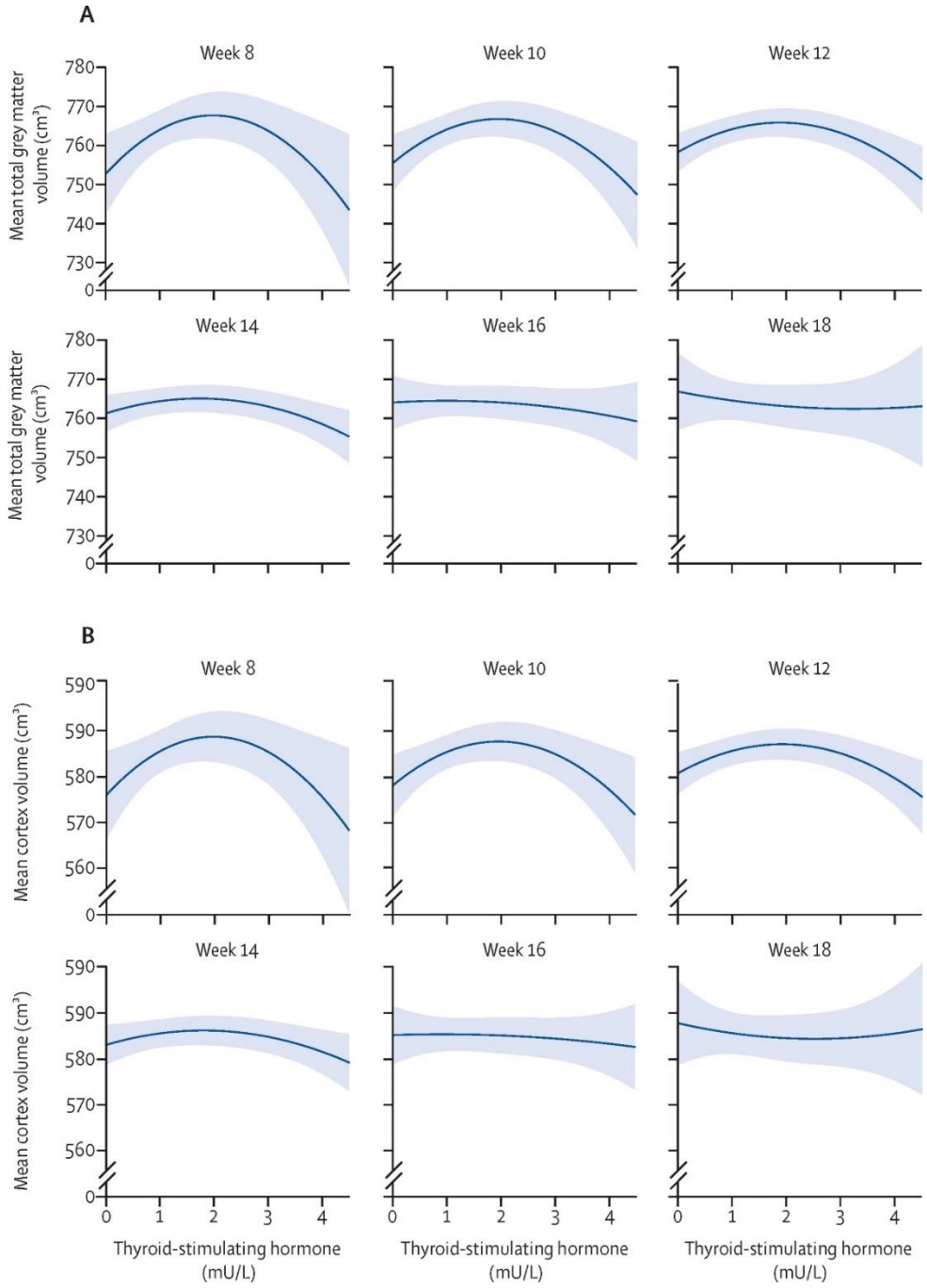


Figure 3.2 Relationship between maternal TSH and offspring grey matter volume in relation to timing of blood sample in gestation (Jansen et al., 2019).

The association of maternal thyroid stimulating hormone with child grey matter volume (A) or cortical grey matter volume (B) stratified by gestational age at blood sampling. Analyses were adjusted for gestational age at blood sampling, maternal age, ethnicity, education level, smoking, thyroid peroxidase antibody positivity (>60 IU/mL), child sex, age at MRI, and total intracranial volume.

Canadian group

The Canadian group studied a group of twenty children whose mothers were known to have had overt hypothyroidism (HYPO) inadequately treated or newly diagnosed during their pregnancy. Although the case-control studies were much smaller than those from the Generation R studies, the included HYPO group had been exposed to a greater degree of maternal thyroid hormone deficiency. The scans were initially undertaken to investigate differences in hippocampal structure (Willoughby et al. 2014a, 2014b), but were re-analysed for corpus callosum (Samadi et al., 2015) and cortical thickness volumes (Lischinsky et al., 2016). Several mothers did not have TSH results available for the first trimester; in such cases their second-trimester values were used to replace missing data. The control group's mothers did not have any thyroid function results.

Hippocampal volumes

Offspring of HYPO mothers had smaller hippocampal volumes bilaterally, the difference being most marked in the right posterior and left anterior segments (Willoughby et al., 2014b). These differences became non-statistically significant when one outlier result was removed, maintaining only a weak trend. In Willoughby et al. (2014a), which had the addition of a group of CH children, HYPO offspring had smaller hippocampal volumes when compared to both CH and controls, but the relationship did not maintain statistical significance when subject to mixed factor ANCOVA. There was no difference in intracranial volumes between children exposed to maternal hypothyroidism and controls.

Corpus Callosum

Children born to HYPO mothers had a smaller anterior corpus callosum (CC) (p 0.019) and genu (p 0.059), and a larger posterior CC (p 0.018) and splenium (p 0.045) (Samadi et al., 2015).

Cortical Thickness

Cortical thickness measurements were performed using a hypothesis-free approach, comparing HYPO offspring to controls, and mild versus severe HYPO subgroup analysis (Lischinsky et al., 2016). Maternal TSH values appeared to predict the degree of thinning and thickening within multiple brain regions. The association

between maternal TSH and cortical thickness is greatest when maternal hypothyroidism occurs earlier in gestation. Some of the correlations remained even when the two most severe cases of maternal hypothyroidism were removed. The authors stated they presented unsmoothed data, as all associations otherwise disappeared. The data analysis approach did try to reduce Type 1 errors by only reporting group-comparison results at $p<0.001$, with the significant results being presented in the table 3.1 below.

Table 3.1 Effect of maternal hypothyroidism on cortical thickness (Adapted from Lischinsky et al. 2016).

Cortical regions showing differences between HYPO (n = 20) and control (n = 24) groups, $p<0.001$. Adapted from Lischinsky et al. 2016. HYPO, mothers with overt hypothyroidism during gestation.

Hemisphere	**Cortical thinning compared to controls**	**Cortical Thickening compared to controls**	**Cortical thinning in severe compared to mild HYPO**	**Cortical thickening in severe compared to mild HYPO**
Left	Insula, fusiform gyrus	Rostral middle frontal gyrus, superior frontal gyrus, superior frontal gyrus, superior parietal gyrus, postcentral sulcus	Medial, orbital sulcus, fusiform gyrus	Precentral gyrus, precuneus gyrus, superior occipital sulcus
Right	Frontomarginal sulcus. Inferior parietal gyrus, Precuneus gyrus	Temporal pole, inferior temporal gyrus, pericalcarine sulcus	Orbital gyrus	Precentral gyrus, lateral orbital sulcus

Summary of results for maternal thyroid dysfunction

Seven studies on MTDF were included, all using T1-weighted MRI, divided between two research groups, with children aged 7 to 14. The Canadian group focused on children exposed to overt maternal hypothyroidism, while the Generation R cohort studied mostly normal thyroid function with a small proportion exposed to subtle dysfunction. Both groups found maternal thyroid function impacted offspring grey matter development, but results conflicted on whether TSH or fT4 was responsible. Interestingly, never both parts of the thyroid axis. In the Generation R cohort, the impact on grey matter volume occurred at either end of normal range of thyroid function, suggesting that there is narrow sweet spot for maternal thyroid function, as it mild excess of maternal thyroid hormones can have a similar impact as mild deficiency. Severe hypothyroidism had the greatest effect (Lischinsky et al., 2016), while in the Generation R cohort, deviations at either end of normal thyroid function also affected grey matter volume (Korevaar et al., 2016; Jansen et al., 2019). The earlier in gestation the exposure to MTDF, the greater the effect (Lischinsky et al., 2016; Jansen et al., 2019).

3.3.2 Congenital Hypothyroidism

Eleven studies examined the effects of congenital hypothyroidism (CH) on neuropsychological development. Five were structural MRI studies, three fMRI studies, three used Magnetic Resonance Spectroscopy (MRS) and one diffusion tensor imaging (dTI) (Cooper et al., 2019). Studies came from Canada, India, Italy, Turkey and the United Kingdom. The seven Canadian CH studies were from the same research group that had published the neuroimaging studies related to MTDF as outlined above, with six purely investigating CH, the seventh study comparing participants with CH to those exposed to maternal thyroid dysfunction and controls (Willoughby, 2014a). All were of a case-control design, with majority treated from the neonatal period. Quality assessment scores ranged between 4 and 8, mainly due to vague description of recruitment and definition of the control groups. It is likely that controls would have had normal thyroid function, as CH should have been detected at newborn screening, but not all countries have routine neonatal screening programmes for CH and thyroid function was often not rechecked when recruited, so thyroid dysfunction cannot entirely be ruled out.

Congenital hypothyroidism: T1- weighted studies

Structural abnormalities:

The majority of the included CH structural studies are those from Canada. Brain MRI scans examined by paediatric radiologists reported no increased incidence of structural abnormalities in children with CH (30) compared to controls (38), mean age 12.5±1.6 years; most abnormalities found were deemed to be not clinically significant (Rachmiel et al., 2013). No morphological differences were reported in neonatal scans at the time of diagnosis with CH (Siragusa et al., 1997; Akinci et al., 2006). Structural data was also available from MRS studies of subjects diagnosed late in childhood, or even adult life. These reported some atrophy in the frontal and parietal lobes (Gupta et al., 1995), or cerebellar atrophy (Jagannathan et al., 1998), although both studies were small, with a total of 10 participants each.

Cortical thickness

Clairman et al. (2015) compared cortical thickness with typically-developing controls, investigating whether there was any association with severity of initial hypothyroidism and/or neuropsychological function. It was the largest congenital

hypothyroidism study included (41 CH: 42 controls), combining archived MRI scans from previous studies (Wheeler et al. 2011 & 2012). Participants were scanned at a mean 12.4 (±1.8) years of age (range 9.3 to 16.8 years). Recognising the increased risk of Type 1 error, due to the number of regions compared, the authors tried to address this by setting a more stringent statistical significance threshold of $p<0.001$. Compared to controls, the children and adolescents with CH exhibited both cortical thinning and thickening in multiple regions of both hemispheres, with several regions showing significance ($p<0.001$). Some findings were bilateral and others observed in just one hemisphere. Some brain regions displayed a relationship between initial disease severity, based on T4 and TSH levels at time of initial diagnosis and/or the aetiology of CH. In relation to cognitive abilities, those children with CH and cortical thickening of frontal, temporal and occipital regions appeared to have reduced test scores.

Areas of cortical thinning were predominantly found in the frontal, parietal and temporal lobes (Clairman et al., 2015). Areas of thickening were more widespread, occurring throughout the cortex, including the occipital lobes, and primarily within sulci. Bilaterally, the temporal poles were thinner in those born with CH compared to controls, as were the right middle and superior frontal sulci. The right middle frontal sulcus was thinner in those born with a higher TSH (i.e. more severe CH). The right superior frontal sulcus was thinner in those with lower T4 concentrations (i.e. more severe CH). In contrast, the left middle frontal sulcus was thicker in those born with lower T4. The cingulate sulcus was bilaterally thicker in those born with a higher TSH level. The left calcarine sulcus was thicker in children with CH and to those with less severe CH, i.e. higher T4 and lower TSH values.

Additional subgroup analysis compared athyreosis (n=13) and ectopic (n=20) aetiologic subgroups. Those born with athyreosis have complete absence of a thyroid gland, whilst those born with an ectopic gland may have some residual thyroid hormone production. The authors found that the right occipital pole was significantly thinner in the athyreotic group ($p=0.0002$). The main difference found in the athyreotic group was a greater amount of thickening in both hemispheres relative to those with ectopic glands: in the left middle frontal gyrus ($p=0.049$), collateral transverse sulcus ($p=0.026$), right occipito-temporal gyrus (two regions, $p=0.009$ and

p=0.003), superior occipital gyrus (p=0.0002), intraparietal sulcus (p=0.0066) and inferior parietal gyrus (p=0.015).

Hippocampal volumes

Three studies examined the potential effect of CH on hippocampal volumes, one comparing CH and controls only (Wheeler et al., 2011), one comparing CH with both children born to mothers with hypothyroidism (HYPO) and a control group (Willoughby et al., 2014a), and one study investigating neuroplastic effects of music lessons in children with and without CH (Zendel et al., 2013). Hippocampal size was not associated with early TH levels, age of starting treatment or aetiology of CH.

Children with CH without music lessons had smaller hippocampi compared to controls, whereas CH with music lessons were similar to controls (p= 0.001), with the right hippocampus even being slightly larger than controls on average (p=0.04) (Zendel et al., 2013). Unlike in the control group, the age of children born with CH was not related to hippocampal size. No differences were found between the two control groups, i.e. with or without music lessons. Although the two CH groups were similar in other variables, there is a risk of substantial bias with those having music lessons self- or parent-selecting, which may be based on potentially confounding, but unrecorded, reasons. The authors suggest the reason that differences are seen between CH groups but not controls, in regards to the effect of music lessons, is that abnormally developed hippocampi are more sensitive to external factors, and a greater amount of music lessons may be needed before structural effects can be seen in controls.

Congenital hypothyroidism: fMRI studies

The three published case-control fMRI studies of children with treated CH were of similar sizes (28 to 29 total) and aged between 8 to 10 (Blasi et al., 2009) and 11 to 15 years old (Wheeler et al., 2012, 2015). TSH and fT4 levels were available for the CH subjects, but not the control groups. The two Canadian fMRI studies used the same subjects, with the two fMRI scans undertaken on the same day. One evaluated visuospatial memory tasks (VST) (Wheeler et al., 2012) and the other tested verbal associated memory tasks (VAM) (Wheeler et al., 2015). The two studies using fMRI to analyse response to VST found a greater amount of activation in adolescents with

CH compared to controls (Blasi et al., 2009; Wheeler et al., 2012). The memory recall results deviated, with one study reporting no differences (Wheeler et al., 2012) and the other reporting reduced correct recall in the CH group (Blasi et al., 2009).

Visuospatial memory tasks

In Blasi et al. (2009), the CH group had greater activation in response to VST of their bilateral supplementary motor area (SMA), bilateral opercular regions of the precentral gyrus, left adjacent insular and the left somatosensory parietal cortex to a significantly greater degree than controls ($p < 0.001$). The controls had higher activation in the left inferior parietal cortex and the anterior intraparietal sulcus extending into the superior parietal cortex.

Wheeler et al. (2012) reported higher TSH levels were associated with increased magnitude of hippocampal activation relative to the control group for recall of both object and place ($p < 0.01$). In addition, whilst the control group mainly activated their left-sided hippocampus, the CH group had bilateral hippocampal activation. Those with the most severe hypothyroidism at diagnosis had the greatest activation, but this was of borderline statistical significance (p 0.05). Higher initial TSH correlated with greater bilateral activation for both Place and Object conditions, with lower fT4 levels, signifying more severe hypothyroidism, related to the extent to which the left hippocampus was active during the object condition.

Verbal associative memory tasks

Minimal differences were found in verbal associative memory tasks, both in fMRI and behaviour (Wheeler et al., 2015), but controls appeared to recruit bilateral hippocampal activation in verbal tasks, whereas it was the CH group in the VST study that had bilateral hippocampal activation (Wheeler et al., 2012).

Congenital hypothyroidism: MRS studies

The three MRS studies were small, case-control studies, ranging from between 3 to 8 CH cases with age-matched controls. Two studies involved those diagnosed late with CH, ages ranging from 6 to 31 years old (Gupta et al., 1995; Jagannathan et al., 1998). The cases were presumed to have CH rather than primary hypothyroidism

developing later in childhood, due to their very low IQ scores and growth restriction. Akinci et al. (2006) identified neonates with CH within the first few days of birth.

Apart from age at time of diagnosis, the three studies are fairly consistent in their design and overall findings. Each study imaged participants newly diagnosed with CH just before levothyroxine was started, with follow-up imaging after 6 to 8 weeks of treatment. The two studies from India, with participants diagnosed very late (6-31years), included additional follow-up imaging at 3 to 6 months post initial scan (Gupta et al., 1995; Jagannathan et al., 1998). Control subjects also underwent repeat imaging, but details were lacking regarding their thyroid function results, recruitment and demographic details.

Two studies reported reduced NAA/Cr (*N*-acetyl aspartate/ creatinine) ratios in pre-treated CH subjects: lower in the cerebellum in all 3 subjects in Jagannathan et al. ($p < 0.01$), with similar findings in the thalamus, and in the parietal white matter (WM) and thalamus in Akinci et al. (2006) ($p<0.05$). No difference in metrics concerning NAA/Cr were found in the study of Gupta et al. (1995). Significantly raised Cho (choline) or Cho/Cr ratios pre-treatment were reported in two studies, Gupta et al. (1995) and Jagannathan et al. (1998) respectively, but did not reach statistical significance in Akinci et al. (2006). Any differences found in the studies disappeared on the post-treatment scans 6 to 8 weeks later. In the smallest study of only 3 affected subjects (Jagannathan et al., 1998), one 14-year-old patient was found to have a high lipid peak on MRS pre-treatment. The scan was repeated to check the finding before treatment was started and found a similar result. By 6 weeks of treatment, the lipid peak had gone.

Congenital Hypothyroidism: Diffusion Tensor Imaging studies

One included study examined white matter microstructure MRI in children with severe CH, using 3.0T diffusion MRI (Cooper et al., 2019). Seventeen children with severe CH (i.e. undetectable fT4 at birth), but diagnosed and treated early with levothyroxine replacement, and twenty controls, underwent DTI scans and hearing and communication ability testing at a mean age of 9.66 years (SD 2.26, range 6.6-15.4). Conventional DTI was used, together with a spherical mean technique (SMT)

method to reduce the interference of fibre orientation effects and produce a microscopic fractional anisotropy (μFA), intra-neurite volume fraction and transverse microscopic diffusivity.

All analyses were controlled for age, gender and IQ. Significant differences were defined as $p < 0.05$ with family wise error correction. In patients with CH, FA was significantly decreased in the cerebellum, bilateral thalami and right temporal lobe. Radial diffusivity (RD) was correspondingly increased in the cerebellum and thalami. No differences were found in MD (mean diffusivity) or AD (axial diffusivity). Correlations were also found between the FA and RD results and listening, language and communication difficulties found in children with severe CH, including in the posterior sections of the corpus callosum, the isthmus, genu and splenium.

The SMT model found significantly lower microscopic FA, intra-neurite volume fraction and increased transverse microscopic diffusivity in CH children compared to controls. The areas affected were again the cerebellum and thalamus, and additionally in the occipital lobe, areas of the CC and white matter adjacent to the sensorimotor cortex, especially in the left hemisphere. Children with severe CH may have significant brain white matter microstructural differences despite early diagnosis and treatment initiation. These changes appear to correlate with difficulties in communication and hearing, although the CH group as a whole had results within normal limits.

Summary of results for congenital hypothyroidism

Children with treated congenital hypothyroidism (CH) showed no significant clinical structural abnormalities compared to controls (Siragusa et al, 1997; Akinci et al., 2016; Rachmiel et al., 2013). In small studies of those diagnosed late in childhood, some had frontal or cerebellar atrophy (Gupta et al., 1995; Jagannathan et al., 1998). TSH appeared to have the greatest effect of degree of cortical thinning or thickness differences in those with CH, as did the aetiology of the CH (Clairman et al., 2015).

Studies consistently report that children with congenital hypothyroidism (CH) perform worse on memory tasks than non-CH controls (Rovet, 1999). The included

neuroimaging studies did not consistently find hippocampal volume differences statistically significant. Children with CH who engaged in music lessons had larger hippocampal volumes, suggesting greater brain plasticity or the influence of environmental factors associated with musical training (Zendel et al., 2013). Children born to mothers with overt hypothyroidism during pregnancy showed the most significant reduction in hippocampal volume compared to children with CH and controls. In fMRI memory studies, there was greater activation of hippocampi in children with CH (Wheeler et al., 2012), and in some other brain areas (Blasi et al., 2009), potentially reflecting compensatory mechanisms.

The few, small MRS studies all reported significant differences compared to controls pre levothyroxine treatment, which resolved post treatment initiation (Akinci et al., 2006; Gupta et al., 1995; Jagannathan et al., 1998). The one CH DTI study found significant white matter microstructural differences in several areas, including the corpus callosum, that correlated with difficulties in communication and hearing (Cooper et al., 2019).

3.3.3 Thyroid function in premature infants

Transient hypothyroxinaemia has been noted after birth in premature infants, and often referred to as THOP (transient hypothyroxinaemia of prematurity). The reasons behind the temporary fall in fT4 are not known, but are likely a combination of loss of the transfer of maternal thyroid hormones whilst the premature infant's own thyroid gland is not fully mature, and that thyroid function is often suppressed during acute illness and stress. Premature infants often require significant medical interventions and support. Children who were born prematurely have increased risk of neurodevelopmental issues (Bhutta et al., 2002). Previous studies have reported thyroid function may influence some of these developmental measures (Ng et al., 2004).

Three THOP studies met the inclusion criteria of this review, two used dTI (Ng, 2014; Hung, 2018), one T1-weighted MRI (Scratch et al., 2014). The quality of the studies was good, two scoring 9/9 stars (Ng 2014, Scratch 2014). The third study scored 8 out of 9 stars, losing a point due to the high rate of attrition for imaging (Hung et al., 2018). Comparison of results is complicated by the studies only measuring one part of the thyroid hormone axis: fT4 was measured by Ng et al. (2014) and Scratch et al. (2014), and TSH measured by Hung et al. (2018).

Thyroid function was checked in the first two weeks of life, with developmental assessments undertaken at pre-school age (Ng et al., 2014; Hung et al., 2018) or in primary school (Scratch et al., 2014). Two studies were able to acquire >40 useable scans each: one followed up children born prematurely with 3.0T structural scans undertaken at 7.5 years of age (Scratch, 2014), the other imaged a subset of premature babies included in the TIPIT trial using diffusion MRI (1.5T) (Ng, 2014). The TIPIT trial (Thyroxine supplementation In Preterm InfanTs) (Ng et al., 2013) randomised premature neonates born before 28 weeks' gestation (mean 25.8 weeks) with THOP to levothyroxine supplementation or placebo until 32 weeks' gestational equivalence, with separate consent obtained to image a subset of neonates when they reached full term equivalence of 40 weeks' (Ng et al., 2014).

The third THOP study was by Hung et al. (2018) using diffusion MRI to image premature neonates born between 23 and 35 weeks' gestation, when they reached full term equivalence. The number of useable scans was small, with only 8 participants included. Many parents did not consent to the neuroimaging component of the study due to use of sedation (Hung et al., 2018). The TIPIT trial was successful in their recruitment, as by using a swaddling technique and no sedation for scans, parents were happier to consent.

No relationship was found between brain volumes at age 7.5 years and fT4 over the first 6 weeks of birth in children born less than 30 weeks (Scratch et al, 2014). Statistically significant associations were found between higher fT4 during the first 14 days after birth and poorer cognition scores at age 7 years with reduced performance in verbal learning ($p=0.02$), verbal memory ($p=0.03$) and simple reaction tasks ($p<0.001$). The authors estimated that thyroid function accounted for 6.5 to 13% of variability in these tasks. The study had the longest follow-up period out of the three studies, together with the most time points for measured fT4 levels.

The dTi imaging used in two of the THOP studies both reported correlations between thyroid hormone levels and markers of white matter microstructural integrity and organisation, such as axial diffusivity (AD) and fractional anisotrophy (FA), at both extremes of the thyroid function range (Ng et al., 2014; Hung et al., 2018). Hung et al. (2018) found that TSH at birth was inversely correlated to AD ($\lambda 1$) in the splenium of the corpus callosum ($p <0.05$) and positively correlated to FA in the anterior limb of the internal capsule ($p <0.01$), but once an outlier was removed, any relationship was lost. No adjustment was made for gestational age, despite a lower gestational age being associated with higher TSH levels.

In the TIPIT trial, no significant differences were found in the primary analysis between the treated and placebo group dTI results (Ng et al., 2014). However, when comparing the highest quartile of fT4 in supplemented babies with the lowest quartile of fT4 results in the placebo group, statistically significant differences were found in several brain regions. LT4 treated babies with fT4 in the highest quartile had evidence of greater white matter organisation than those with very low plasma fT4 concentrations in the placebo group.

DTI metrics within the TIPIT trial group found babies with the fT4 in the lowest quartile compared to those in the upper 3 quartiles of the placebo group (9:10) demonstrated lower FA values in all eight brain regions, but none reached statistical significance. The opposite was seen with ADC values (apparent diffusion coefficient), with babies in the lowest fT4 quartile having higher ADC values in the posterior CC (p 0.05). Streamlines through all brain regions were reduced in both length and number, reaching statistical significance in the right posterior limb of the internal capsule (p 0.02). The treated group in the highest quartile of fT4, compared to the lower three quartiles in the treated group, had lower ADC values in seven out of eight brain regions, with higher FA in six out of eight brain regions. This was again reflected in the number and length of streamlines being greater in those with fT4 in the highest quartile of the LT4-supplemented group. The only area to reach statistical significance for both ADC and FA was the anterior CC.

Summary of results for THOP

Three studies of children born prematurely, with THOP. Two scanned whilst neonates, one when 7.5 years old. None included both fT4 and TSH values. Two studies measured fT4 during the neonatal period. One found no relationship between fT4 and brain volumes (Scratch et al., 2014), but did report associations with neurodevelopmental assessments. In the TIPIT RCT, there was no difference between the levothyroxine-treatment and placebo group neuroimaging results. However, there was a higher degree of white matter organisation in those in the highest quartile of fT4 in the treated group compared to those with lowest fT4 levels in the placebo group (Ng et al., 2014). The third reported correlations of MRI metrics with TSH, but none reached significance (Hung et al., 2018). The three studies conflicted on the association between thyroid function and neurodevelopmental outcomes.

Table 3.2 Included studies in systematic review.

Study (Author, Year, Country)	Type of study	MRI modality & Field strength (Tesla)	Exposure	Age when scanned*: mean (SD), range	Age at thyroid sampling/diagnosis: mean (SD), range	Size (useable scans): if case-control exposed : non-exposed	Male sex (percentage) split into case:control if appropriate)	Study quality using NOS
	Maternal thyroid dysfunction							
Ghassabian et al. 2014, Netherlands	Case control	Structural MRI 3.0T	Population cohort study. Lowest 5% maternal fT4 defined as hypothyroxinaemic.	7.9 (1.0), NA	13.5 (2.0) gestational wk, 5.1-17.9 gestational wk	27 : 625	50:50	9
Willoughby et al. 2014a, Canada	Case control	Structural MRI 1.5T	HYPO	10.1 (0.52), 9-12 years	First/second trimester	19 : 30	63:48	5
Samadi et al. 2015, Canada	Case control	Structural MRI 1.5T	HYPO	10.6 (0.78), 9-14 years	During trimester 1, 2 & 3	20:22	68:68	5
Korevaar et al. 2016, Netherlands	Cohort	Structural MRI 3.0T	Population cohort,	8.0^, 95% range 6.2-10.0 years	13.^2 wk, 95% range 9.8-17.5 wk	598	49	9
Lischinsky et al. 2016, Canada	Case control	Structural MRI 1.5T	HYPO	10.3 (0.65), 10 – 12 years	First/second trimester+	20 : 24	64:63	5

Study (Author, Year, Country)	**Type of study**	**MRI modality & Field strength (Tesla)**	**Exposure**	**Age when scanned*: mean (SD), range**	**Age at thyroid sampling/diagnosis: mean (SD), range**	**Size (useable scans): if case-control exposed : non-exposed**	**Male sex (percentage) split into case:control if appropriate)**	**Study quality using NOS**
Jansen et al. 2019, Netherlands	Cohort	Structural MRI 3.0T	Population cohort	9.9^, IQR 9.7-10.2 years	13.1^ wk (gestation), IQR 12.1-14.5	1981	49	9
	Maternal thyroid dysfunction and children born with congenital hypothyroidism							
Willoughby et al. 2014b, Canada	Case control	Structural MRI 1.5T	HYPO, CH and those with no known thyroid dysfunction	10.7 (1.71), 10-14	First/second trimester+	16 HYPO : 22 CH : 26 controls	65:44:35	5
	Congenital hypothyroidism							
Blasi et al. 2009, Italy	Case control	fMRI	CH : no CH	9.3 (0.73), 8 - 10 years	Neonatal	15 : 13	No data	7
Clairman et al. 2015, Canada	Case control	Structural 1.5T	CH : no CH	12.4 (1.8), 9 - 16 years	Neonatal	41 : 42	48:48	6
Jagannathan et al. 1998, India	Case control	Structural & MRS 1.5T	CH* : no CH	8, 14, 31 years	8,14,31 years	3 : 7	No data	4
Rachmiel et al. 2013, Canada	Case control	Structural 1.5T	CH : no CH	12.5 (1.6), 10-15 years	Neonatal	30 : 38	47:46	6
Wheeler et al.	Case control	Structural 1.5T	CH : no CH	12.1 (1.7), 9-15 years	Neonatal	35 : 44	40:57	6

Study (Author, Year, Country)	**Type of study**	**MRI modality & Field strength (Tesla)**	**Exposure**	**Age when scanned*: mean (SD), range**	**Age at thyroid sampling/diagnosis: mean (SD), range**	**Size (useable scans): if case-control exposed : non-exposed**	**Male sex (percentage) split into case:control if appropriate)**	**Study quality using NOS**
2011, Canada								
Wheeler et al. 2012, Canada	Case control	fMRI	CH : no CH	13.4 (0.99), 11 -15.5 years	Neonatal	14 : 15	57:53	6
Wheeler et al. 2015, Canada	Case control	fMRI	CH : no CH	13.4 (1.0), 11.5-14.7 years	Neonatal	14 : 12	57:57	6
Zendel et al. 2013, Canada	Case control	Structural 1.5T	CH : no CH	11.36 (1.5), No range data	Neonatal	15 : 15	48:48	6
Siragusa et al. 1997, Italy	Case control	Structural 1.5T	CH : no CH	22 days	Neonatal	11 : 22	18:no data	8
Akinci et al. 2006, Turkey	Case control	Proton MRS	CH : no CH	5-7days and 8 weeks (post thyroxine in CH)	Neonatal	8 : 8	No data	5
Gupta et al. 1995, India	Case control	MRS	CH* : no CH	6-15 years	6-15 years	5 : 5	No data	7
Cooper et al. 2019, UK	Case control	DTI	CH : no CH	9.7 years (2.3), 6.2–15.4	Neonatal	17 : 20	39:48	7
	Premature infants							

Study (Author, Year, Country)	**Type of study**	**MRI modality & Field strength (Tesla)**	**Exposure**	**Age when scanned*: mean (SD), range**	**Age at thyroid sampling/diagnosis: mean (SD), range**	**Size (useable scans): if case-control exposed : non-exposed**	**Male sex (percentage) split into case:control if appropriate)**	**Study quality using NOS**
Ng et al. 2014, UK	Randomised case control trial	DTI 1.5T	THOP : randomized to levothyroxine treatment or placebo.	Full-term equivalence.	Born prematurely before 28 weeks. Median 25.8 weeks (24 – 27+1)	23 : 19	No data	9
Scratch et al. 2014, Australia	Cohort	Structural 3.0T	Cohort	7.5, 6.8–8.3 years	Birth	52	37	8
Hung et al. 2018 Taiwan	Cohort	DTI 1.5T	Cohort	Full-term equivalence.	Born at 23 to 35 weeks' gestation, with TFTs in first 24 hours of birth, scanned at full-term	8	48 (no data for subgroup imaged)	8

*Mean age of the group exposed to abnormal thyroid function. ^indicates median value. * delayed diagnosis and treatment initation.

Abbreviations: wk, week; DTI, diffusion tensor imaging; fMRI, functional magnetic resonance imaging; MRS, magnetic resonance imaging; IQR, interquartile range; HYPO, maternal hypothyroidism; NOS, Newcastle-Ottawa Scale; TFTs, thyroid function tests.

3.4 Discussion

The aim of this review was to identify all published, peer-reviewed human MRI neuroimaging studies of participants that had been exposed to abnormal thyroid function during gestation or childhood. The hope was to identify structures within the brain that may be most sensitive to abnormal thyroid function and any neuroimaging evidence for key timepoints during neurodevelopment.

The search terms were broad, but did identify several studies not found during my initial literature review, including all three THOP studies. The twenty-two included studies naturally divided into three types of childhood exposure to abnormal thyroid function: MTDF, CH and THOP. However, the majority of the studies were from the just two research groups, often re-analysing the same participants' scans, risking increasing any potential recruitment bias. Structural T1-weighted MRI was the most commonly used MRI modality, and was the sole modality used in the MTDF studies. The remaining included studies used other MRI techniques: fMRI, MRS or DTI. Almost all studies scanned children of school age, with the exception of four that scanned neonates (Siragusa et al., 1997; Akinici et al., 2006; Ng et al., 2014; Hung et al., 2018) and a CH study including MRI data of an adult with a very late diagnosis of CH (Jagannathan et al., 1998). Included studies varied in their measurement of thyroid hormones, with some including both TSH and fT4 values, others fT4 or TSH alone.

Although the included studies differed in brain regions examined and type of MRI imaging used, several studies in all three sub-groups reported neuroimaging differences related to thyroid hormone exposure in brain structures such as total grey matter volume, hippocampal volume and activity, corpus callosum, cerebellum and thalamic metrics. There were conflicting results of either TSH or fT4 reported as having correlation with neuroimaging or cognitive measures, usually without a reciprocal relationship with the other.

No significant gross structural brain abnormalities were reported, including a study that had paediatric neuroradiologists examine the scans of children with CH and controls (Rachmiel et al., 2013). The two very small studies of late-diagnosed CH reported mild atrophy in cortical (Gupta et al., 1995) or cerebellar (Jagannathan et

al., 1998) regions compared to controls. MTDF and CH studies reported differences in grey matter volumes, but varied in brain regions reported and conflicted as to whether TSH or fT4 was associated. Interestingly, despite TSH and fT4 being part of the same axis, in studies that measured both hormones only one parameter was significant. Corpus callosum volumes were the most commonly reported white matter volume, but other white matter regions were often not included. Similar to previous animal studies, there did appear to be effects of thyroid function on hippocampal volumes or in functional MRI, but the number of studies were small. There were metabolic changes pre- and post-treatment of those diagnosed late with CH, including signals that can be associated with myelination. Diffusion tensor imaging allows a more detailed assessment of white matter structure, but only three dTI studies were found, one in children with CH and two in THOP.

Gross structural

The majority of studies did not expressly evaluate for the presence of gross structural abnormalities. It was only evaluated in a few CH studies. The lack of significant abnormalities, even in the few cases of late diagnosis of CH, should not be surprising. In the early 1990s, two imaging studies of people with cretinism causing severe neurological disabilities found few differences compared to controls. There were some abnormalities in the basal ganglia in both studies. The first was a CT imaging study including cases of neurological and myxoedematous (now called CH) cretinism (Halpern et al., 1991), the second comprising three case reports of neurological cretinism with MRI imaging (Ma et al., 1993). Neither met the inclusion criteria of this review, but offer interesting findings, especially the CT imaging study, as it included 50 scans of severe cases. Calcification of basal ganglia was found in 15 out of the 50 subjects, but only in those with severe hypothyroidism and did not correlate with severity of neurodevelopmental abnormalities (Halper et al., 1991). The Ma et al. (1993) study reported T1 hyperintensities and T2 hypointensities in the globus pallidum and substantia nigra of the three affected cases. Both studies' subjects had motor abnormalities that were similar to other extrapyramidal disorders, but the clinical findings were the same as those with normal appearing basal ganglia.

Effects on grey matter

Grey matter and cortical regions were examined in all three subgroups of study. Thyroid function did appear to have effects on grey matter in those studied. However, there were conflicting results about whether TSH or fT4 had an effect, complicated by fact that several studies did not include data on both parts of the axis. Interestingly, in those that did report both TSH and fT4, only one half of the axis appeared to have an effect. The cerebral cortex specifically refers to the outer 6 cell layers of grey matter on the outer surface of the brain. Grey matter contains the neuronal bodies, but in contrast to the cortex, can include various structures that include some fibre tracts, and subcortical structures (Stiles & Jernigan, 2010). Because both the cerebral cortex and the subcortical nuclei contain the cell bodies of neurons they are grey in appearance, thus giving rise to the term “grey matter”. The cerebral cortex is a 2–5 mm thick layer of cells that lies on the surface of the brain (the word cortex comes from the Latin term meaning bark, as in the bark of a tree). In cross-sections of the brain, the cortex is a thin, grey strip following the surface of the brain. The subcortical structures are deeper and are termed nuclei; they are formed by clusters of neurons that form centres that relay signals between different areas of the cortex and body.

Only two research groups investigated MTDF. The Generation R studies were the largest included MTDF studies, with the majority of maternal thyroid function results in the normal range. The Canadian studies were much smaller than the Generation R studies, but the group of controls were larger and cases had a greater degree of MTDF as they had been exposed to overt maternal hypothyroidism *in utero*. The initial Generation R study compared offspring born to mothers with hypothyroxinaemia to the rest of the cohort (Ghassabian et al., 2014). The definition used for hypothyroxinaemia was arguably too broad, defined as fT4 in the lowest 5% of the cohort, and still resulted in a small number of cases (27/625). In an earlier study reporting reduced IQ in children born to mothers with overt hypothyroidism, it was only those in the highest 0.3% of TSH that demonstrated any effect (Haddow et al., 1999). No significant effect on IQ was found in much larger studies with stricter definitions of suboptimal gestational thyroid function (Lazarus et al., 2012; Casey et al., 2017; Hales et al., 2018).

To address the potential issue of the artificial lowest 5% of fT4 cut-off, Korevaar et al. (2016) reanalysed with thyroid function as a continuous variable, including TSH as well as fT4. Initially it was fT4, not TSH, that demonstrated an effect on grey matter volume at either end of the normal range of maternal thyroid function. However, when only including participants with both fT4 and TSH results available, this changed to have TSH, not fT4, as having a significant association with grey matter volumes, forming an inverted U-shaped curve (Jansen et al., 2019). Repeating this analysis with a larger group of slightly older children, again an inverted U-shaped relationship was found between grey matter volume and fT4 (Jansen et al., 2019). The relationship was lost once corrected for total intracranial volume. However, it is debatable whether correcting for intracranial volume is needed or could indeed obscure correlations, as it is potentially a related variable to grey matter volume. Low birth weight and increased risk of premature delivery is a recognised complication of both hypo- and hyperthyroidism (Millar et al., 1994; Blazer et al., 2004). There are conflicting results regarding head size, with some studies reporting smaller infant head circumference in those born to mothers with an element of thyroid hormone deficiency (Shields et al., 2011; Su et al., 2011), and others reporting CH children have larger head circumference (Ng et al., 2004).

Using archived scans of children with CH and controls, cortical thinning was mainly confined to areas in the frontal, parietal and temporal lobes, with cortical thickening occurring throughout the cortex and primarily within sulci (Clairman et al., 2015). The study also included sub-group analysis between children with different aetiologies of CH; those with no thyroid gland had a greater amount of cortical thickening in both hemispheres, compared to others with TH deficiency but still some intrinsic production. A similar technique was used by Lischinsky et al. (2016), although they compared exposure to mild and severe maternal hypothyroidism *in utero*. Similar to observations in CH, children born to mothers with poorly controlled hypothyroidism had the greatest differences in cortical thickness (Lischinsky et al., 2016). The difficulty is interpreting what these differences represent clinically.

Timing of thyroid hormone deficiency

Sub-analysis looked at the potential effect of gestational timing of when the MTF results were taken (Jansen et al., 2019). Those with TSH at either end of normal

range at week 8 had the greatest statistical relationship with grey matter volume, gradually decreasing with each gestational week until significance was lost from week 14 onwards. There were no serial MTF measurements, hence the study was limited by availability of only one time point for each participant and thus no option to consider potential trajectories over pregnancy. It highlights that if future studies provide evidence that intervention is required, any potential intervention would be difficult, as effects seem to be greatest early in pregnancy, before usual antenatal care has begun.

The peri-natal period also appears to be a vulnerable time for exposure to other types of thyroid dysfunction. CH studies suggest that permanent brain development can still be affected by TH deficiency towards the end of pregnancy. Those born with the most severe form of CH, i.e. no functioning thyroid gland, despite early detection and treatment and the supply of maternal fT4 *in utero*, have an increased risk of lower scores in several areas of executive function (Razón-Hernández et al., 2022).

Specific brain regional effects

Due to the hippocampi and corpus callosum being common brain structures affected by thyroid deficiency in animal studies (Desouza et al., 2005), and reported issues with memory in CH (Rovet, 1999) and MTDF (Pharoah and Connelly, 1991), the Canadian group focused on these areas in both MTDF and CH. The corpus callosum findings are discussed in the white matter section below.

Hippocampal volumes

As predicted, children with CH had smaller hippocampal volumes. One study compared the effects of three groups on hippocampal volume: children exposed to maternal hypothyroidism had the smallest hippocampi compared to CH or controls (Willoughby et al., 2014a). The greater impact on hippocampal volumes in MTDF could potentially be because, unlike the CH children, the children exposed to maternal overt hypothyroidism were exposed from the very start of the first trimester, rather than peri-natal period.

Another CH study found that CH children who had regular music lessons had normalisation of hippocampal size (Zendel et al., 2013). No such difference was

found between controls without CH who did or did not have music lessons. The authors suggest that abnormally developed hippocampi in children with CH may be more sensitive to external factors. The length and amount of music lessons were not reported, so it is unknown if children with CH may find learning an instrument harder, and so have to practice a greater amount to achieve similar results to controls, and therefore stimulating more neuroplasticity, i.e. the brain's ability to adapt and modify (Voss et al., 2017). Another explanation could be that children with less severe CH, which may have less effect on hippocampal size, find music lessons easier than those with severe CH, and are therefore more likely to continue with lessons. The study did not assess for hearing impairment (Zendel et al., 2013), which is common in children with CH.

In untreated cretinism, 50% had hearing loss to varying degrees, some severe (Halpern et al., 1991). In patients with CH who were treated early, almost a quarter of children studied still had hearing impairment (Cooper et al., 2019). This confirms that thyroid function later in gestation is still important for later neurodevelopment. The human foetus starts to respond to auditory stimuli *in utero* around 24-25 weeks', mirroring the time cochlear myelination begins to develop. The impact of hearing impairment on brain development/ MRI findings, is still not fully understood (Ratnanather, 2020). With early hearing loss, it has been postulated that decreased input from the thalamus to the auditory cortex in hearing impairment can manifest in diverted inputs to other cortical areas (Ratnanather, 2020).

Effects on white matter

Few of the MTDF studies included results of white matter tracts outside of the corpus callosum. The anterior corpus callosum was found to be smaller in children exposed to maternal hypothyroidism, with the posterior corpus callosum larger (Samadi et al., 2015). White matter and myelination changes are found in foetal rats exposed to TH deficiency (Morte et al., 2002). It is unclear whether the lack of white matter volumetric data is due to study design or lack of significant results resulting in less reporting. Any white matter changes in humans may be on the microstructural level, and difficult to appreciate/detect on standard structural MRI scans. Another

explanation is that greater differences are found in grey matter than white matter. The TH receptor alpha is expressed in grey matter earlier in foetal development than the beta TH receptor which is involved in myelination from the second trimester onwards (Bernal et al., 2005).

Three DTI studies did examine white matter and white matter microstructure in cases of CH (Cooper et al., 2019) or THOP (Hung et al., 2018; Ng et al., 2014). As metrics of DTI can be impacted by a variety of factors, they are difficult to interpret, especially as statistical significance was lost once an outlier was removed. Further studies examining the corpus callosum are needed, as it is a key area of myelination and animal models have demonstrated changes in size and architecture in those exposed to TH deficiency (Bernal et al. 2015).

In children with the most severe forms of CH, but receiving prompt treatment whilst neonates, there were significant reductions in FA and a corresponding increase in RD in the cerebellum and bilateral thalami (Cooper et al., 2019). Further modelling found a similar pattern in the white matter in the occipital lobe, areas of the CC and white matter adjacent to the sensorimotor cortex, mostly in the left hemisphere. These results could indicate a decreased density and/or increase in orientation dispersion of white matter fibres, creating a disturbance in the white matter microstructure of participants with CH. The neuroimaging findings correlated with difficulties in hearing and communication assessments, albeit the CH group as a whole had communication results within normal range.

The two THOP studies using DTI presented data from the corpus callosum in premature babies (Ng et al., 2014; Hung et al., 2018): The small study by Hung et al. (2018) found TSH at birth was negatively correlated with AD (λ1) in the splenium of the corpus callosum ($p < 0.05$) and positively correlated with FA in the anterior limb of the internal capsule (ALIC) ($p < 0.01$). Lower AD can suggest axonal damage or reduced white matter integrity, with higher FA indicating the opposite. In the larger study, the TIPIT trial, babies in the supplemented group with highest quartile of fT4 had higher FA values and longer and more numerous streamlines in the anterior CC. The length and number of streamlines in the lowest quartile of fT4 in the placebo group were lower in the majority of brain regions, suggesting poorer white matter

organisation, but did not reach statistical significance. In DTI studies of white matter, streamlines visualise the predominant directions of water diffusion, reflecting the pathways of white matter tracts. They facilitate tractography, mapping neural connections, and help infer connectivity patterns between brain regions. Streamlines also indicate the structural integrity of white matter, with disruptions suggesting possible damage or abnormalities.

In the THOP studies, there are multiple factors present in prematurity affecting brain development. Therefore, evaluating the contribution of thyroid function is difficult, especially as thyroid hormone levels are often a barometer for illness or stress. An earlier THOP study utilising cranial ultrasound in premature infants found twice the increased risk of echolucency in cerebral white matter in those with the lowest quartile of fT4 (Leviton et al., 1999). The study adjusted for body size and degree of prematurity and found the association remained between lower fT4 and greater risk of echolucency, a marker of white matter damage. The exact link to causation or mechanism was not confirmed, but the authors postulated that THs could be neurologically protective against the insults premature infants are exposed to, e.g. poor cerebral blood flow or hypotension. The finding that foetal fT4 increased with the gestational age at which the baby was born, lends support to the hypothesis that children with CH are exposed to TH deficiency in the last half of gestation, as they are unable to start producing their own fT4.

The TIPIT trial paper included in this systematic review was the nested neuroimaging component (Ng et al., 2014). A later paper included some of these participants as well as non-imaged subjects, who were later followed-up at 42 months of age with neurodevelopmental assessments (Ng et al., 2020). It was not included in the systematic review results, as it although it included secondary analysis of MRI metrics, it did not meet quality criteria based on the NOS. No information was included how many participants had scan results, if they were representative of the group etc. The study reported that a secondary analysis found FA at term significantly correlated with cognitive scores at 42 months' of age in the corpus callosum (p 0.027) and frontal right lobe (p 0.045), but only presented three brain regions, and did not include details of the number of regions analysed, making it difficult to assess the risk of type 1 error.

The differences in DTI metrics at the extremes of fT4 in the interventional TIPIT imaging study suggests that thyroid hormone levels are having an effect on white matter development, but effect of clinical intervention with thyroxine supplementation may be limited, or need a much larger number to treat to see significant effects in DTI measures between the treatment and placebo group. The conflicting developmental outcomes between the three THOP studies sounds a cautionary note, with one finding no difference based on TSH at 2 years of age (Hung et al., 2018), one finding improved developmental outcomes at 42 months in those with higher fT4 as a neonate (Ng et al., 2020) and one finding poorer developmental outcomes in those with higher fT4 (Scratch et al., 2014). The conclusion is that not only are larger studies needed, but that supplementing with thyroxine is not necessarily associated with no harm.

Myelination and hearing loss

One study included hearing assessments (Cooper et al., 2019). Sensorineural hearing loss is well documented in both iodine deficiency related endemic cretinism and in CH. The impact on hearing loss on brain development, especially white matter, raises the question about potential cause or effect. Certain auditory experiences can promote and maintain myelin levels in both developing and aging brains (Deoni et al., 2012). Auditory enrichment, such as musical training, correlates with increased white matter connectivity in the corpus callosum, especially in individuals who start musical training in childhood (Bengtsson et al., 2005; Steele et al., 2013). Auditory training, even in aged rats, partially reverses age-related decline in myelin gene expression in the primary auditory cortex (de Villers-Sidani et al., 2010).

Extremely few studies have included hearing assessments for children born to mothers with MTDF. The children of mothers positive for anti-TPO antibodies during pregnancy had significantly higher rate of sensorineural hearing loss (22.7% compared to 4.3%), but thyroid function was not tested for due to lack of sample volumes available (Wasserman et al., 2008). One published study of maternal gestational thyroid function included data about the offsprings' hearing obtained during routine childhood follow-up in Finland, together with parent-rated questionnaires at age 7 years (Päkkilä et al., 2018). No linguistic or sensory

developmental issues were found using parental questionnaires and no statistically significant association between hearing impairment and maternal thyroid function during pregnancy. However, it is unclear what routine examination of hearing included, as no audiometry data was presented. A small study of 9 mothers with subclinical or overt hypothyroidism detected in pregnancy did have audiometry brainstem responses measured compared to controls, and found no significant differences (Radetti et al., 2000).

Brain activity and metabolism

The fMRI and MRS studies were only undertaken in the CH group, not MTDF or THOP. The fMRI studies were conducted in those detected through neonatal screening and started on replacement promptly. The MRS studies were mostly undertaken in those diagnosed late with CH and compared pre- and post-treatment markers of metabolism.

Previous non-imaging studies reported visuospatial task (VST) deficits in children with treated CH (Rovet et al., 1999). Unfortunately, the two studies of VST did not overlap in brain regions studied, but both found children with CH required additional neural resources to perform VS tasks correctly compared to controls and suggest that even a short period of TH deficiency early in life can affect functioning in adolescence (Blasi et al., 2009; Wheeler et al., 2012). Greater severity of thyroid hormone deficiency predicted greater hippocampal activation in the CH group, as well as bilateral hippocampal recruitment (Wheeler et al., 2012). The additional neural activation may help to reduce detectable differences in VST performance. Minimal differences were reported in VAM tasks (Wheeler et al., 2015).

All three CH MRS studies found significant metabolic differences in newly diagnosed CH brains compared to controls before treatment was initiated. Six to eight weeks after starting thyroxine replacement, the differences had resolved. In the included studies, levels of NAA, a marker of neuronal integrity and myelination, increased with thyroxine therapy. NAA is involved in lipid synthesis during myelination, and its deficiency is associated with Canavan disease, a leukodystrophy characterized by impaired myelination due to a deficiency of the enzyme N-acetyl aspartoacylase (Shaag et al., 1995).

Cho/Cr ratios were elevated pre-treatment compared to controls, and rapidly normalised post-therapy in two studies (Gupta et al., 1995; Jagannathan et al., 1998). A similar trend was seen in the third MRS study, but did not reach significance (Akinici et al., 2006), which could reflect the lower age and myelination maturation stage of the participants, as the children were scanned as neonates. Choline is part of the polar head of membranes including myelin. The reduction in Cho post-treatment, in an opposite manner to NAA, may also reflect myelin maturation, with the choline molecule less able to move (Miller, 1991).

Myelin contains lipids, but is usually a highly organised structure, with little lipid signal seen on MRS imaging. However, if myelin is broken down or disrupted, the lipids it contains can be detected. Of the total 16 CH subjects with MRS results, only one participant had a lipid peak. It is therefore difficult to determine its significance. The researchers repeated the pre-treatment scan to confirm the lipid peak (Jagannathan et al., 1998). THs are known to be involved in myelinogenesis (Amur et al., 1984), hence the resolution in the subject's lipid peak post levothyroxine treatment may reflect an effect of thyroxine on the promotion of myelin maturation. In animal studies, experimental hypothyroidism can cause abnormalities in myelinogenesis, but can be reversed by thyroxine replacement in the first 20 days of life (Noguchi et al., 1985).

A retrospective analysis of the largest international cohort of children diagnosed with MCT8 deficiency reported on neurodevelopmental phenotypes and included details of MRI scans of 13 patients (Groenweg et al., 2020). The study, published after the initial systematic review, would not have met the inclusion criteria of this systematic review. The scans, acquired as part of routine care, were assessed retrospectively by a pediatric neuroradiologist, with varying protocols, scanners, ages, and without controls.

The scans, performed at a median age of 8.0 months (range: 5.0-187.0 months), revealed a consistent finding of a global delay in myelination. While myelination improved with age, it had not fully normalised in the oldest patient (15 years). Most cases demonstrated diffuse cortical and subcortical atrophy, dilated ventricles, and widened subarachnoid spaces. Seven patients also had MRS imaging available. In 6

out of 7 patients (85.7%), magnetic resonance spectroscopy (MRS) indicated an increased choline peak and a decreased N-acetyl aspartate (NAA) peak, suggesting aberrant myelination and general atrophy. These findings were corroborated by postmortem examination of an 8-year-old, which showed a global reduction in brain volume and myelination. The effects on myelination appear to be a delayed trajectory of myelination and the reported MRS findings match those of the late diagnosed CH patients pre-treatment reported in the included MRS studies.

Strength and limitations

The strength of this review is that a broad search was undertaken to ensure all appropriate studies of different thyroid hormone abnormalities on the developing brain. The number of titles screened was very large, but it did find several papers I had not found during my comprehensive literature search and makes me confident that no other relevant studies have been missed. Studies were of good quality, but heterogeneous in areas of the brain studied and thyroid hormones measured.

Twenty-two studies at first appears to be a relatively good number, but once the results are divided into the three different conditions, the exposed population size for each condition is relatively small. Few studies overlapped in the brain regions being studied. The total number of participants is also smaller than first appears, as more than one study included analysis of the same archived neuroimaging data; this occurred in the majority of the MTDF and CH studies from Canada, and with two of the three Generation R studies (Ghassabian et al, 2014; Korevaar et al, 2016). The number of children imaged that were deemed exposed to thyroid hormone dysfunction during early life is relatively small and increases the risk that any bias in initial recruitment could be propagated.

The majority of children studied had a mean age between 8 to 12 years old, so allows for some comparison between studies. However, puberty scores were often missing, and puberty is a key point in myelination and white matter development (Genc et al., 2020). Controls in the Canadian MTDF and CH were not always clearly established to have had no exposure to abnormal thyroid levels. However, it can be

presumed that controls in the CH studies had been screened at birth for CH, due to many countries having national screening programmes.

There were disparities between whether TSH, fT4 or both were measured. Due to a wide variety of focus and design between the included studies, no meta-analysis could be undertaken. The majority of structural imaging was undertaken using 1.5T MRI scans, which captures less detail compared to higher strength MRI scanners. It may be when similar studies are undertaken in the future, with higher resolution scans, more significant structural differences could be detected. The majority of the studies focused on thyroid hormone deficiency rather than excess. Three studies did suggest that exposure to thyroid hormones towards the upper end of normal range could have a potentially detrimental effect on neurodevelopment (Korevaar et al., 2016; Jansen et al., 2019; Scratch et al., 2014), with one study finding the opposite for those supplemented with levothyroxine (Ng et al., 2014; Ng et al., 2020).

Future studies

Future studies should measure both TSH and fT4. Although several of the included studies reported associations with fT4 or TSH, often only one was found significant and it was difficult to compare between studies as many only measured one hormone, fT4 or TSH. Including hearing assessments would also be of interest, as non-imaging studies in CH have established significantly increased risk of hearing impairment, as have DIO 2 and 3 deficient mouse models (Ng et al., 2017). Very few studies have looked for potential impacts of MTDF on offspring hearing.

Longitudinal neuroimaging studies could provide insight into potential effects on brain development and trajectories. For children with CH, by including thyroid function results measured at several time points during gestation and during childhood, it could help answer questions about effect of childhood thyroid function and/or replacement targets during childhood. No imaging studies of adults or young adults affected by exposure to abnormal thyroid function during gestation or childhood were found in the searches undertaken for this systematic review. Imaging adults would reduce the variables associated with age, growth and pubertal development during rapid time of neurodevelopmental changes during childhood and adolescence.

Due to differences in brain structure appearing to be relatively subtle, even in those exposed to severe and prolonged thyroid deficiency since childhood, more detailed structural MRI results and increased use of functional and diffusion MRI could help explore differences at a microstructural level. Future diffusion tensor MRI analysing white matter tracts using both hypothesis-free approaches and those based on *a priori* knowledge from animal and human data could help build on current understanding of pathways implicated in neurodevelopmental disorders.

There are hints that excess thyroid function as well as thyroid hormone deficiency could affect child development. Investigating the effects of high fT4/low TSH on neuroimaging findings would be of interest. Investigating hyperthyroidism is more difficult, as it is much less common than hypothyroidism, but potential future studies could attempt to recruit women of childbearing age who are treated for thyroid cancer who are purposely over-treated with levothyroxine in order to suppress TSH and follow them prospectively.

3.5 Conclusion

This systematic review is the first of its kind in this topic area. By design, its search criteria were broad to identify all types of thyroid dysfunction that a study participant could have been exposed to during childhood or gestation. The systematic search identified three types of thyroid dysfunction: MTDF, CH, and THOP. In all three groups of included studies, several papers reported some differences related to thyroid hormone function. Results were conflicting, as some studies reported relationships between neuroimaging findings and TSH levels, while in others, the association was stronger with fT4. In studies including both TSH and fT4 data, often only one of the two was significantly associated with imaging findings, although one would expect that any associations observed would be reciprocal.

The first trimester appears to be the most sensitive to MTDF. However, deficiencies in thyroid hormones later in gestation or during the neonatal period still have long-term behavioural and cognitive impacts, as seen in children born with CH and begun early on treatment in previous non-imaging studies (Rovet and Hepworth, 2001). Due to many of the included studies' results not yet being replicated by other research groups, and with many trends losing statistical significance once adjusted

for potential confounders or outliers, this review cannot confidently conclude any specific effects of TH on neuroimaging findings. However, the trends appear consistent between similar studies, and there are strong indications that the first trimester is when the foetus is most sensitive to derangements in maternal thyroid function.

A small number of the included studies suggest that exposure to excess thyroid hormone may also cause deleterious effects (Korevaar et al., 2016; Jansen et al., 2019; Scratch et al., 2014), mirroring previous associations between excess TH and increased risk of ADHD in large behavioural, non-imaging studies (Andersen et al., 2014), and increased hearing dysplasia in neonates exposed to higher maternal fT4 (Su et al., 2011).

Future studies on the potential effects of abnormal thyroid function exposure during gestation or childhood should include both TSH and fT4 measurements. Follow-up imaging in adulthood, along with longitudinal imaging during childhood, could help determine if thyroid function affects developmental trajectories or causes permanent neuroimaging changes. The heterogeneity of MRI methods and the brain regions studied in the included research limits confidence in interpreting the results. Future studies should aim to address this variability in their design.

Chapter 4

Chapter 4. Controlled Antenatal Thyroid Screening Study III: Effects of gestational thyroid status on adolescent brain morphology

4.1 Introduction

The current chapter focuses on the effects of maternal thyroid dysfunction during gestation on offspring morphological brain development in adolescence. Neuroimaging was undertaken as part of the CATS III study, recruiting a subset of the original CATS II (controlled antenatal thyroid screening) study cohort (Hales et al., 2018). The CATS II study was a follow-up of the original CATS cohort (Lazarus et al., 2012). The CATS study was the first randomised control trial looking at the effect on the IQ of offspring at age 3 post intervention of treating or not treating maternal suboptimal gestational thyroid function (SGTF) with levothyroxine. The CATS II study followed a subset up at age 9 with a wider range of assessments and included children exposed to normal GTF or SGTF (untreated or treated) (Hales et al, 2018 & 2020).

4.1.1 Suboptimal gestational thyroid function

As outlined in Chapter 1, thyroid function is critical for neurodevelopment. During gestation, maternal free T4 is the only thyroid hormone able to cross the placenta (Calvo et al., 1990; Arrojo e Drigo, 2011). Suboptimal gestational thyroid function (SGTF) is an umbrella term referring to any abnormality in maternal thyroid function during pregnancy. SGTF includes both overt and subclinical hypothyroidism or hyperthyroidism (although much less common than hypothyroidism), as well as isolated maternal hypothyroxinaemia, i.e. low free T4 but normal TSH.

It is still unknown which specific areas of the brain are most affected by SGTF. Animal studies suggest potential micro- and macro-structural effects, but little human data are available. Several observational studies have reported detrimental effects on offspring neurodevelopment of a lack of maternal T4, including reduced IQ, hearing loss, memory and motor disorders (Pop et al., 2003, Pharoah et al., 1976, Korevaar et al., 2016). Studies of potential effects of maternal thyroid function on offspring behaviour suggests that there may be subtle effects on neurodevelopment even with relatively minor changes in maternal thyroid function, for both hypo- and hyperthyroidism (Fetene et al., 2017).

Modern MRI techniques allow us to examine morphological changes in the human brain in unprecedented detail. The previous chapter (chapter 3) provided a

systematic review of the current published MRI neuroimaging studies which had investigated the effect of exposure to abnormal gestational thyroid hormone levels on neurodevelopment. Only a small number of neuroimaging studies investigated effects of either MTDF (maternal thyroid dysfunction), congenital hypothyroidism (CH) or transient hypothyroxinaemia of pregnancy (THOP). There was heterogeneity in design and focus of the studies. There were conflicting results across studies, with some showing associations between neuroimaging findings and TSH levels, while others found stronger links with fT4. Notably, the first trimester appears to be the most sensitive period for MTDF, although deficiencies in thyroid hormones later in gestation or during the neonatal period also appear to have long-term behavioural and cognitive impacts, with majority of evidence from children with CH. A few studies indicated that excess thyroid hormone exposure may also have harmful effects (Jansen et al., 2019; Scratch et al., 2014), aligning with previous findings linking high TH levels to increased risk of a ADHD diagnosis or traits (Andersen et al., 2014 and 2018; Fetene et al., 2017; Hales et al. 2020)

4.1.2 Maternal thyroid function and effect on offspring IQ: origin of the controlled antenatal thyroid screening (CATS) study

The published studies on the effect of maternal thyroid dysfunction on offspring IQ are conflicting. A study published in 1999 found children born to mothers with severe hypothyroidism (i.e. TSH at or above the 99.7% centile) had a reduction in IQ of 4 points compared to the control group, but the p value was borderline significant at p 0.06 (Haddow et al., 1999). When the hypothyroid group was subdivided into those on levothyroxine treatment (14/62), albeit suboptimal in view of high TSH, and those on no treatment (48/62), there was a significant difference in IQ, with children in the untreated group having a mean IQ score 11 points lower (p 0.005), but IQ was still in normal range. It provoked increased discussion in the field about whether there should be routine, universal antenatal screening of pregnant women for thyroid dysfunction. Chapter 1 illustrates how varied the type and degree of thyroid abnormalities can be, as well as the physiological changes that occur during pregnancy.

To investigate the effect of SGTF on IQ, two very large RCTs were undertaken (Lazarus et al., 2012; Casey et al., 2017). They both used the lowest 2.5% of fT4 and TSH above the 97.5% centile as their cut offs for defining SGTF. Neither found

significant differences in IQ between children born to mothers with SGTF treated or untreated with levothyroxine supplementation (Lazarus et al., 2012, Casey et al., 2017).

4.1.3 Evidence for impact of maternal thyroid function on other aspects of neurodevelopment

There are only a small number of MRI studies to have examined the association between maternal thyroid status and offspring brain structure. Two MRI studies examining the effect of maternal thyroid function on offspring brain development found an inverted U-shaped relationship between grey matter volume and either end of normal range of fT4 (Korevaar et al., 2016) or TSH (Jansen et al., 2019). Jansen et al. (2019) hypothesised that there would be a greater difference earlier in gestation. After creating subgroups based on the gestational age at which the mothers' bloods were tested, an inverted U-shaped association of maternal TSH with child total grey matter volume and cortical grey matter volume was demonstrated. This pattern was most apparent at week 8 and gradually decreased at each gestational week until it lost significance from week 14 onwards. Jansen et al. (2019) re-analysed the earlier data in Korevaar et al.'s study (2016), only including subjects with *both* fT4 and TSH values. They found that the previous relationship between grey matter volume and fT4 was no longer significant, with the TSH correlation reaching statistical significance instead.

In addition to effects on brain morphology, human population studies have observed an increased risk of ADHD following *in utero* exposure to either subclinical or overt hypo- or hyperthyroidism (Andersen et al., 2014, Modesto et al., 2015). Attention deficit hyperactivity disorder (ADHD) is a common neurobehavioral disorder characterised by overactivity, inattention and impulsivity. The origins of this disorder are unclear but the intrauterine environment, including maternal thyroid status, may play an important role. Gestational hypothyroidism in rats leads to altered offspring behaviour and abnormal flow of information to the hippocampus and somatosensory cortex (Navarro et al., 2015), potentially mediated via hypomyelination of white matter tracts (Morreale de Escobar et al., 2004, Friesema et al., 2004). Thyroid hormone receptor β-deficient mice show significantly higher exploratory activity and reduced habituation compared with their wild-type counterparts (Ookubo et al., 2015), whilst transgenic mice harbouring a human mutant thyroid hormone receptor

(TRbeta1), who are euthyroid except for a short period postnatally, also manifest the core features of ADHD (Siesser et al., 2006), suggesting that even temporary disturbances in thyroid balance can elicit sustained neurobehavioral effects. These observations mirror the high prevalence of ADHD reported in patients with resistance to thyroid hormone due to TR beta mutations (Brucker-Davis et al., 1995)..

4.1.4 Controlled Antenatal Thyroid Screening (CATS) study

The current study follows on from the original Controlled Antenatal Thyroid Screening (CATS) studies, CATS (Lazarus et al., 2012) and CATS II (Hales et al., 2018, Hales et al., 2020). The CATS (Controlled Antenatal Thyroid Screening) study was a large (>15,000 pregnant women in South Wales) randomised controlled trial which studied the effect of thyroid hormone replacement on offspring IQ, initially at age 3. The randomisation was on whether the TFTs taken when recruited into the study at their 12 week antenatal appointment were processed immediately, or stored to be analysed post birth. If randomised to immediate analysis and found to have SGTF, mothers were started on levothyroxine.

At the time of publication of both RCT studies, CATS I (Lazarus et al., 2012) and Casey et al. (2017), the lack of difference in IQ results between the untreated and treated SGTF groups were thought to be that mild SGTF may have had an effect on IQ, but the intervention may have occurred too late in gestation to cause a difference between untreated and treated groups. The CATS II study aimed to address this by following up children at age 9, this time including those from the normal GTF group as well as the SGTF treated and untreated groups (Hales et al., 2018 & 2020). A wider range of cognitive assessments were undertaken, looking at both IQ and a wider range of neurodevelopmental markers, including ADHD traits.

The CATS II study had 452 participants, split into three groups: untreated SGTF, treated SGTF and normal GTF (Hales et al., 2014). There was no difference in offspring IQ between the groups, with the addition of no significant difference confirmed between the normal or SGTF groups. Another difference to the CATS I analysis is that in CATS II, in secondary analysis, the treated group was subdivided into optimally treated and overtreated. No difference was found in IQ in this subanalysis, but differences were found on ADHD traits.

The overtreated group was an inadvertent result of starting a relatively high dose of levothyroxine in the original CATS trial. The levothyroxine treatment dose was not based on maternal weight, rather the starting dose was the same for all participants in the treatment group, starting at a comparatively high dose (150 micrograms). The mothers started on levothyroxine had repeat TFTs at approximately 20 and 30 weeks' gestation, and doses adjusted as indicated. However, the relatively high starting dose of levothyroxine resulted in almost one-third of treated women developing free T4 levels >97.5th percentile of the entire CATS cohort when results were repeated at 20 and/or 30 weeks' gestation. Application of three validated instruments to assess child behaviour (strengths and difficulties questionnaire [SDQ], child ADHD questionnaire, social communication questionnaire [SCQ]) (Goodman, 1997, Thapar et al., 2000, Lord and Rutter, 2003) showed that scores above clinically relevant thresholds for conduct and hyperactivity were significantly more prevalent in children exposed to both over-treatment or overt hypothyroidism (Hales et al., 2020).

4.2 Hypothesis

In this thesis, I hypothesised that abnormal thyroid hormone bioavailability during neonatal development alters brain maturation and morphology. The main objective of the CATS III study was to investigate this by applying novel neuroimaging readouts and technologies to a subgroup of the CATS cohort. Part of the imaging protocol included acquisition of T1 data, in addition to DTI and qMT. I have analysed the T1 data for this thesis.

This chapter explores whether abnormal TH bioavailability during foetal development affects brain maturation on a macrostructural level. The microstructural component of the CATS III study is not included and is an ongoing piece of work. The CATS III study is the first imaging study to include a significant number of children whose mothers had SGTF, some of whom were treated with levothyroxine during gestation, including a subset with exposure to excess T4.

4.3 Methods

4.3.1 Study design and participants

The current study is a follow-up of the Controlled Antenatal Thyroid Screening (CATS) studies, CATS I (Lazarus et al., 2012) and CATS II (Hales et al., 2018, Hales et al., 2020, Muller et al., 2020). The original CATS study recruited approximately 22,000 women in the UK and Italy between June 2002 and May 2006 (Lazarus et al., 2012). Women were screened at a median of 12 weeks 3 days of gestation and randomised to either screen or control groups; both provided serum samples at recruitment with the screen group having their thyroid function tested immediately and the controls after their child was born. Women with a fT4 concentration < 2.5th percentile and/or TSH > 97.5th percentile were classified as having suboptimal gestational thyroid function (SGTF); percentiles were calculated from the CATS cohort. Women in the screening group with SGTF were treated with levothyroxine (starting dosage 150 µg) for the remainder of their pregnancies. Dosages were adjusted where necessary, to maintain a serum TSH of 0.1 to 1.0 mIU/L, following measurement of TSH and FT4 at 6 weeks post-treatment initiation and at 30 weeks' gestation. Treated mothers whose TSH became suppressed, were defined as the overtreated, whilst those whose TSH stayed at 0.1 or above were deemed optimally treated. SGTF women diagnosed after delivery were advised to visit their general practitioner for further management.

The primary outcome of CATS I was the children's IQ at 3 years of age, among 390 (303 in the UK) treated (screen) and 404 (306 in the UK) untreated (control) mothers (assessors were blinded to treatment group). A further 20 789 (15 593 in the UK) women made up the normal-GTF group. The CATS II study revisited >400 of the CATS children for testing of IQ, behaviour, anthropometric and cardiometabolic status at 9 years of age (Hales et al., 2018, Hales et al., 2020, Muller et al., 2020).

For the current study, mothers who had previously provided consent to be contacted about future research studies were recruited from the CATS II database. We sought to recruit mother-child pairs from each of the 4 study groups (normal gestational thyroid function [normal GTF]: untreated SGTF; optimally-treated SGTF [fT4 and TSH within reference range at 20 and 30 weeks' gestation]; over-treated SGTF [fT4 >17.7 pmol/l at either 20 or 30 weeks' gestation as defined by the top 2.5th percentile

in the entire CATS UK cohort at consent into the trial]) (Hales et al., 2020). To avoid selective recruitment and the potential for bias, we initially sought to identify all potentially eligible individuals and randomly selected subjects to be approached to take part, adjusting for any imbalance between groups with respect to age and sex as recruitment progressed. Subjects were recruited between November 2016 and February 2020. Charlie Hales and Raghav Bhargava both contributed to the initial recruitment and scanning before I joined the CATS III trial. I recruited and scanned the majority of the included participants. Full research governance approval was received from Cardiff University (reference SPON 1502-16) and Wales Research Ethics Committee 1 (16/WA/0237). Written and informed consent was obtained from the parents, and assent from the children. Information sheets and consent and assent paperwork are available in the appendix.

4.3.2 Eligibility and investigational measurements

Adolescents were eligible for inclusion if they were aged between 10-16 years and if they lived sufficiently close to enable travel to and from the Cardiff University Brain Research Imaging Centre (CUBRIC) within one day. Subjects with metalwork with the potential to affect MRI scans, including non-removable metal dental braces or piercings, a pacemaker or other implanted devices, were excluded from participation. After informed consent, a safety screening questionnaire was completed before they entered an initial 'mock' MRI scanner to allow for acclimatisation. One subject decided against proceeding with the study after this. Since pubertal status was a potentially important confounder on brain maturation, all participants completed a validated pubertal self-assessment questionnaire based on the Tanner classification (Taylor et al., 2001). This gives a combined score from a two-part pictorial questionnaire, rated 1 to 5 for each relevant body area; higher scores correlate with more advanced pubertal development.

4.3.3 Imaging

Images were collected on a Siemens Healthineers PRISMA 3T scanner using a 32-channel receive-only radiofrequency head coil, using an inversion recovery fast spoiled gradient recalled sequence (repetition time = 2300 ms, echo time = 3.06 ms, inversion time = 850 ms, flip angle = 9^{O} , pixel bandwith = 230 kHz, matrix 288 x 256, isotropic resolution $1mm^3$).

4.3.4 Image processing and quality assurance

T1-weighted images were processed and analysed using the FreeSurfer (Fischl, 2012) pipeline and automated segmentation (FreeSurfer Version 6.0; https://surfer.nmr.mgh.harvard.edu/). The FreeSurfer image analysis suite constructs a representation of the cortical surface through automated segmentation using the Desikan Killany atlas and produces global and regional measures (area and volume) of cortical and subcortical areas (Fischl, 2012) (see figure 2.3 in chapter 2). To account for possible movement artefact and processing error, image quality assessment was performed using two approaches: 1) after images were processed, I visually assessed the cortical surface images, with all deemed useable; 2) the ENIGMA Cortical Quality Control Protocol 2.0 (April 2017) (Stein et al., 2012) (http://enigma.ini.usc.edu/) was used to objectively assess the quality of the FreeSurfer outputs, with all rated as “good”.

4.3.5 Statistical analysis

Statistical analyses were performed using R statistical software, version 4.3.1 (Team, 2021). The analysis scripts are included in the appendix, and are available online on the Open Science Framework (DOI 10.17605/OSF.IO/PRKU8).

Age, sex and pubertal status have all been shown to affect brain maturation during adolescence (Genc et al., 2020, Herting and Sowell, 2017). Consequently, analyses were adjusted for child sex, child age at time of MRI scan and self-rated Tanner pubertal score. For completeness, I have presented results both with (partial correlation) and without (full correlation) adjustment of covariates.

Data were inspected to establish whether they met assumptions for parametric testing; where assumptions were violated, non-parametric tests were used. Missing data were treated in one of two ways: 1) where a variable was treated as a covariate (e.g., total Tanner [pubertal] score), missing data were replaced with the median score for the rest of the cohort (note that the ages of participants with missing Tanner scores were close to the median age of the cohort, hence replacing missing puberty score data with the median of the whole cohort was deemed appropriate); 2) where a variable was a variable of interest (dependent or independent variable), participants with missing data were excluded from the analysis for that test only.

Outliers were identified using the interquartile range criterion; observations > $IQ_{.75}$ + 1.5*IQR or < $IQ_{.25}$ - 1.5*IQR were deemed outliers. Where outliers were detected, we present results excluding these observations.

Primary analyses: effects of SGTF and treatment on global brain structure

The effect of group (normal GTF; untreated SGTF; optimally-treated SGTF; and over-treated SGTF) on global measures of brain volume (including total grey matter volume, cortex volume, cerebral white matter volume, subcortical grey matter volume and estimated total intracranial volume) was assessed using a one-way analysis of variance (ANOVA) where data met assumptions of normality and heteroscedasticity, or the Kruskal-Wallis test where these assumptions were violated (i.e., for subcortical grey matter data). To account for potential confounders of sex, age and pubertal status, we employed multiple regression.

Secondary analyses: effects of maternal thyroid stimulating hormone (TSH) and free thyroxine (FT4) on global brain structure

The effects of baseline TSH and FT4 levels (measured at entry into CATS I; 12 weeks' gestation) on global measures of brain structure, (including total grey matter volume, cortex volume, cerebral white matter volume, subcortical grey matter volume and estimated total intracranial volume) was assessed using full and partial (accounting for sex, age and pubertal status) correlations. Since TSH and FT4 violated assumptions of normality, non-parametric correlation tests were used. Kendall's Tau was employed for its increased rigidity, beneficial in small samples and when dealing with tied observations in the data.

For those in the treated and overtreated SGTF groups, we also explored full and partial correlations between FT4/TSH levels at 20 and 30 weeks' gestation and the same global measures of brain volume. Values of $p<.05$ were considered statistically significant for all primary and secondary analyses. Given the non-independence of total, cortical and subcortical brain volumes, we did not perform corrections for multiple comparisons on these results; this should be considered when interpreting p values close to the alpha threshold of 0.05.

Exploratory analyses: effects of maternal thyroid stimulating hormone (TSH) and free thyroxine (fT4) on regional brain structure

In exploratory analyses, we investigated the effects of maternal TSH and fT4 levels at baseline on subregional cortical and subcortical grey matter volumes using full and partial (accounting for sex, age and pubertal status) correlations. As this analysis was exploratory, no outliers were removed from the dataset. Bonferroni correction was used to correct for the many multiple comparisons associated with analysing each sub-region of the brain independently.

4.3.6 Sample size

The structural T1 data presented here were acquired as part of a longer scanning protocol that included diffusion tensor imaging (DTI) and quantitative magnetisation transfer (qMT) protocols. The sample size for the study was calculated based on the planned DTI analysis and is not immediately transferable to the T1 section of the CATS III study. Instead, a sensitivity analysis to establish the minimum effect size (f) we would have 80% power to detect, given our sample size of ≥20 participants per group (for our primary analysis). Our sample size provided 80% power to detect medium to large effect sizes (f ≥ .38) in an ANOVA comparing four groups.

Thanks and acknowledgement

Carolyn McNabb provided substantial guidance and support with the approach to statistical analysis and writing the R code used for statistical analysis. Laura Bloomfield and Sonya Foley provided guidance with the pre-processing steps, and Laura Bloomfield assisted in the quality assessment process.

4.4 Results

4.4.1 Demographic characteristics

Figure 4.1 summarises the number of participants at each stage of the CATS studies. A total of 86 adolescents were recruited as part of the CATS III study. One participant was excluded after failing to acclimatise to the mock scanner, leaving 85 participants whose image data were available for analysis (normal GTF = 24, untreated SGTF = 21, optimally treated SGTF = 20, over-treated SGTF = 20), all of whom passed quality assessment for their T1-weighted images.

Demographic characteristics are presented in Table 4.1. Thyroid function test results at time of consent into CATS are presented per group, as well as additional test results at later timepoints for the optimally-treated and over-treated SGTF groups. As expected, median maternal TSH concentrations were higher, and median maternal fT4 concentrations lower, in the three SGTF groups compared to the normal GTF group. Child age, sex and pubertal status were not significantly different between groups. Thyroid function results for each group were similar to those in the CATS II study. Mirroring the CATS II cohort, 95.3% self-reported as white. No statistically significant differences between the four groups were found in the overall IQ measurement of FSIQ (0.95), or in the sub-domains (VMIQ, PRIQ, WMIQ, PSIQ).

Of the 20 women who were over-treated with levothyroxine (fT4 concentration in top 2.5% of the cohort, defined as fT4 >17.7 pmol/l at 20 or 30 weeks' gestation), five had sustained over-treatment throughout the second and third trimesters. The median (IQR) fT4 concentrations at 20 weeks and 30 weeks for the over-treated group were 18.9 pmol/L (17.9, 19.6) and 17.3 pmol/L (16.6, 18.5), respectively, while median (IQR) TSH values at these times were 0.11 mIU/L (0.02, 0.33) and 0.16 mIU/L (0.02, 0.33), respectively. In the optimally-treated SGTF group, the median (IQR) fT4 concentrations at 20 and 30 weeks' gestation were 15.6 pmol/l (13.8, 15.8) and 17.3 pmol/l (16.6, 18.5) respectively, with median (IQR) TSH values of 0.36 mIU/L (0.17, 1.20) and 0.27 mIU/L (0.12, 0.65).

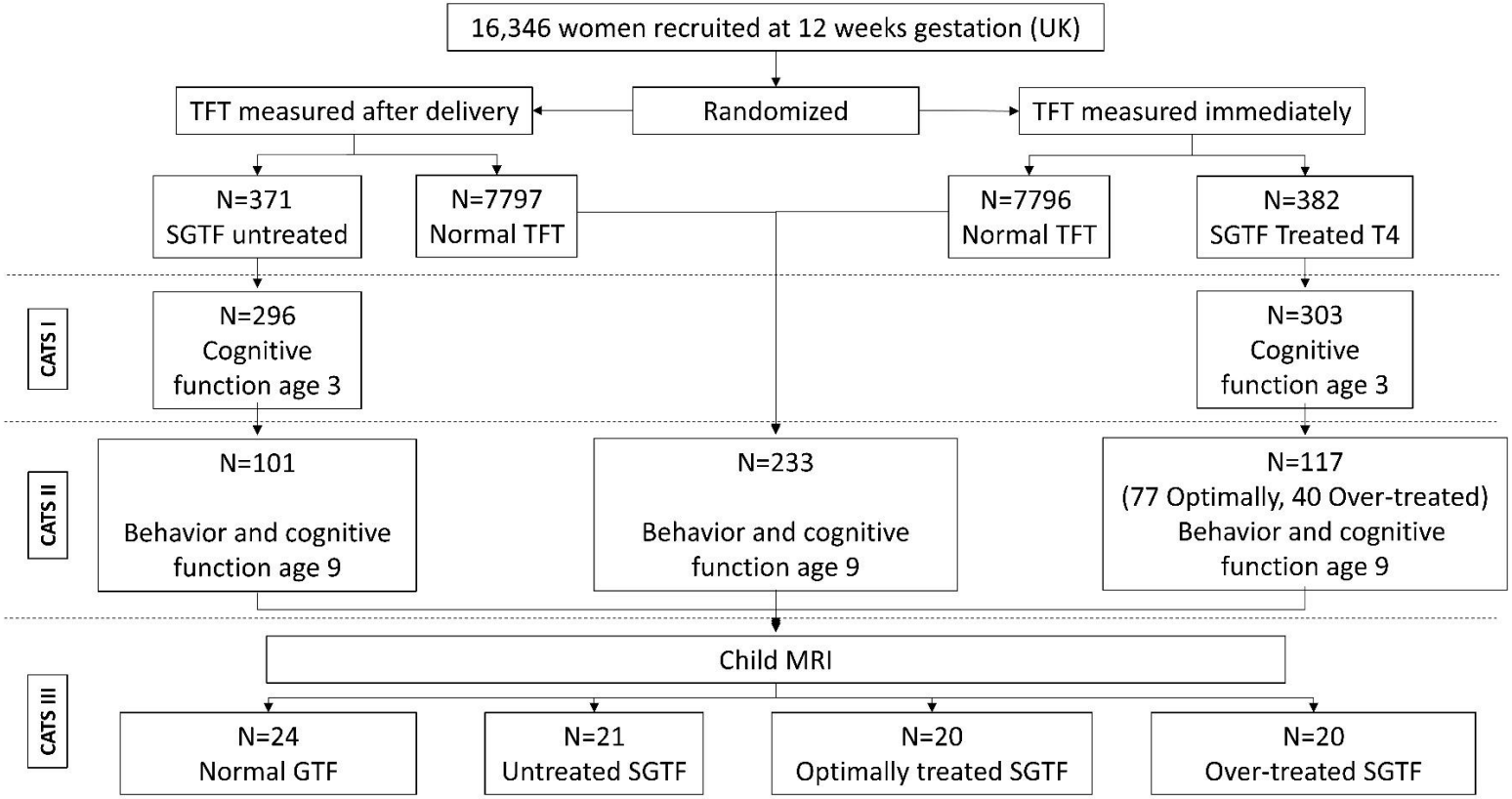


Figure 4.1 Flow of participants through the CATS studies. Illustrates the initial recruitment in South Wales, UK, for CATS-I, when mothers were randomized to screening and treatment for SGTF or not. In CATS-I child IQ was assessed at 3 years of age, the follow-up study, CATS-II, in which child IQ and behaviour was assessed at 9 years of age, and CATS-III when children underwent MRI scans. Abbreviations: CATS, controlled antenatal thyroid screening study; TFT, thyroid function tests; GTF: gestational thyroid function ; SGTF, suboptimal GTF. CATS I (Lazarus et al., 2012) and CATS II (Hales et al., 2020).

Table 4.1. Baseline characteristics of the CATS III study cohort. Abbreviations: GTF= gestational thyroid function, SGTF= suboptimal gestational thyroid function, TSH=thyroid stimulating hormone, fT4=free thyroxine

Characteristic	Normal GTF	Untreated SGTF	Optimally treated SGTF	Over-treated SGTF	Test statistic; P value
	N=24	N=21	N=20	N=20	
TSH at 12 weeks* (study entry; mlU/L)	0.96 (0.49, 1.40)	3.65[a] (0.83, 5.20)	3.71[b] (1.63, 4.53)	4.35[b] (3.87, 5.04)	H=32.0; p<0.001
TSH at 20 weeks (mlU/L)	-	-	0.37 (0.17, 1.20)	0.11 (0.02, 0.42)	W=277.5; p=0.037
TSH at 30 weeks^ (mlU/L)	-	-	0.28 (0.12, 0.65)	0.16 (0.02, 0.33)	W=228; p= 0.161
fT4 at 12 weeks* (study entry; pmol/L)	14.1 (13.7, 14.6)	10.8[b] (10.7, 12.8)	10.7[a] (10.0, 13.6)	12.5 (10.6, 12.8)	H=19.7; p<0.001
fT4 at 20 weeks (pmol/L)	-	-	15.6 (14.6, 16.3)	18.9 (17.9, 19.6)	W=37.5; p<0.001
fT4 at 30 weeks^ (pmol/L)	-	-	14.6 (13.8, 15.8)	17.3 (16.6, 18.5)	W=46.5; p<0.001
Male children, (%)	41.7	52.4	50.0	50.0	H=0.602; p=0.896
Age of children (years)	13.4 (12.4, 15.0)	13.9 (13.1, 14.8)	14.6 (12.9, 15.5)	12.9 (12.5, 13.7)	H=7.52; p=0.057
Pubertal score[τ]	6.0 (5.8, 8.0)	8.0 (5.0, 8.0)	7.0 (6.8, 8.0)	6.5 (5.8, 7.0)	H=5.81; p=0.121

Values are medians (1st and 3rd quartile) unless stated otherwise.
*missing thyroid function tests, n=1
^missing thyroid function tests, n=2
[τ] 4 participants missing total Tanner scores. Data shown here are excluding these participants.
[a]$p<.01$ vs normal GTF; [b]$p<.001$ vs normal GTF (posthoc pairwise Wilcoxon test)

4.4.2 Primary analyses: effects of SGTF and treatment on global brain structure

Global brain tissue volumes are presented in Figure 4.2. There were no significant differences between the four groups in total grey matter volume ($F(3,81)=1.055$, $p=0.373$), cortical volume ($F(3,81)=1.084$, $p=0.361$), cerebral white matter volume ($F(3,81)=0.402$, $p=0.752$), subcortical grey matter volume ($H(3)=1.63$, $p=0.652$) or total intracranial volume ($F(3,81)=0.752$, $p=0.525$). Accounting for potential confounders (sex, age and pubertal status) using multiple regression did not reveal any differences in global tissue volume between groups (data not presented).

4.4.3 Secondary analyses: effects of maternal thyroid stimulating hormone (TSH) and free thyroxine (fT4) on global brain structure.

Baseline levels of TSH (measured at 12 weeks' gestation) were significantly positively correlated with global measures of brain volume (see Table 4.2); however, this effect was not robust to the inclusion of age, sex, and pubertal status in the model (see partial correlation estimates in Table 4.2). No statistically significant effects of baseline fT4 on global measures of brain volume were observed.

Among those whose mothers were randomised to receive treatment with levothyroxine (optimally-treated and over-treated SGTF), no effects of 20 or 30 weeks' gestation levels of maternal TSH or FT4 on global measures of brain volume were observed (table 4.3). Only total grey matter volume was shown to correlate with TSH at 30 weeks' gestation in those who were optimally-treated, but this did not survive correction for multiple comparisons. One participant had overt hypothyroidism, with a TSH of 38 mIU/L, almost four times the next highest TSH result. This participant was excluded from analysis, along with any participant whose brain volumetric measurements met criteria for outlier-based exclusion (see methods section). Results were similar when all data were included.

Although groups were well matched for sex, it was a statistically significant variant in partial correlation calculations. In a previous observational population cohort study, an association between maternal thyroid function and ADHD traits was found in girls, but not in boys (Päkkilä et al., 2014). Therefore, an exploratory analysis was performed to examine if any interaction was found between treatment group and sex.

There was no significant effect of sex on the effect of treatment group on any global brain volume ($p>0.05$).

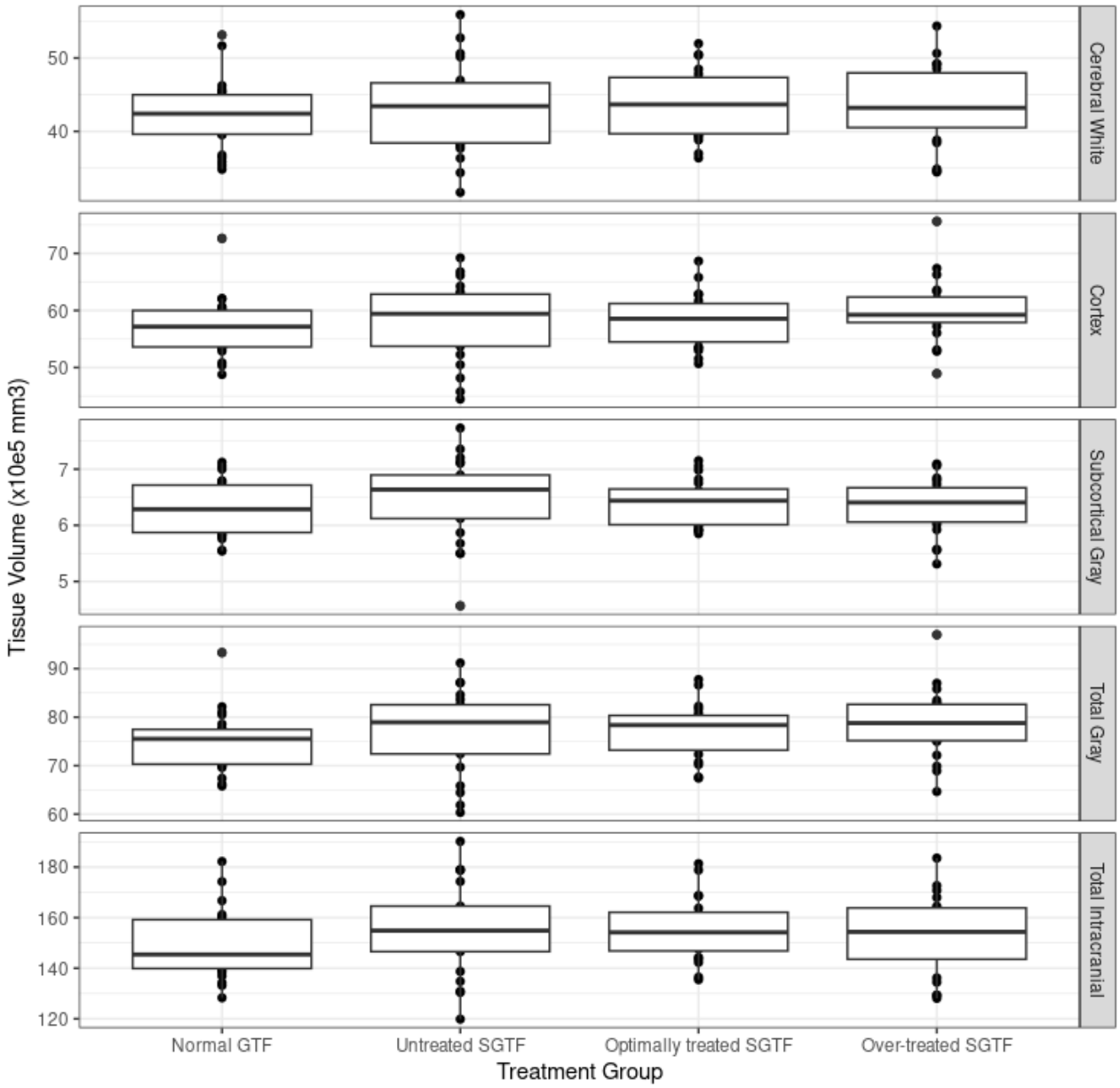


Figure 4.2. Global brain tissue volumes within each treatment group. Boxplots represent medians and interquartile ranges. Abbreviations: GTF= gestational thyroid function, SGTF= suboptimal gestational thyroid function.

Table 4.2. Global tissue effects of baseline (12 weeks' gestation) maternal TSH and fT4 levels.

	Full correlation estimate (Kendall's tau)	**Full correlation p value**	**Partial correlation estimate (Kendall's tau)**	**Partial correlation p value**
Baseline TSH (N= 84)				
Total grey matter volume	0.191	0.011	0.148	0.054
Cortical volume	0.183	0.015	0.140	0.068
Cerebral white matter volume	0.178	0.017	0.137	0.073
Subcortical grey matter volume	0.150	0.046	0.102	0.183
Total intracranial volume	0.172	0.021	0.131	0.085
Baseline fT4 (N= 84)				
Total grey matter volume	0.013	0.862	-0.044	0.560
Cortical volume	0.024	0.753	-0.034	0.652
Cerebral white matter volume	0.039	0.607	-0.004	0.962
Subcortical grey matter volume	0.020	0.789	-0.022	0.776
Total intracranial volume	0.036	0.634	-0.002	0.983

Partial correlation models accounted for sex, age and pubertal status at the time of imaging. Statistically significant correlation estimates are presented in bold. Note that no corrections for multiple comparisons were used for this analysis. Abbreviations: TSH=thyroid stimulating hormone, fT4=free thyroxine.

4.4.4 Exploratory analyses: effects of maternal thyroid stimulating hormone (TSH) and free thyroxine (fT4) on regional brain structures

Exploratory analyses revealed positive correlations between TSH but not FT4 levels at 12 weeks' gestation (baseline) and regional brain volume across several regions, even after accounting for age, sex and pubertal status (table 4.4 & 4.5). However, none survived correction for multiple comparisons using the Bonferroni method (α=0.00008) and so results should be interpreted with care.

The areas with the strongest significant partial correlations were with TSH in the bilateral ventral diencephalon and accumbens regions (table 4.4). Other regions having positive correlation with TSH that survived partial correlation, were the left cortex, bilateral caudate nuclei, and the mid-posterior section of the corpus callosum. In contrast, the only significant negative association with maternal baseline fT4 was only observed with third ventricle volume (-0.177, p=0.019) (table 4.5).

Table 4.3 Global tissue effects of 20 and 30 weeks' gestation maternal TSH and fT4 levels in those whose mothers were randomized to receive levothyroxine.

	Full correlation estimate (Kendall's tau)	Full correlation p value	Partial correlation estimate (Kendall's tau)	Partial correlation p value
20 weeks' gestation TSH (N=35)				
Total grey matter volume (N=33)	0.085	0.493	0.063	0.623
Cortical volume (N=33)	0.070	0.575	0.048	0.712
Cerebral white matter volume	0.204	0.090	0.186	0.134
Subcortical grey matter volume	0.087	0.467	0.058	0.642
Total intracranial volume	0.173	0.150	0.139	0.263
Optimally-treated patients only (N=18)				
Total grey matter volume	0.197	0.255	0.091	0.637
Cortical volume	0.223	0.197	0.125	0.515
Cerebral white matter volume	0.249	0.150	0.186	0.334
Subcortical grey matter volume	0.144	0.404	0.017	0.930
Total intracranial volume	0.236	0.172	0.179	0.353
Over-treated patients only (N=16)				
Total grey matter volume	0.201	0.292	0.307	0.144
Cortical volume (N=15)	0.161	0.418	0.287	0.194
Cerebral white matter volume	0.289	0.130	0.366	0.082
Subcortical grey matter volume	0.236	0.216	0.310	0.140
Total intracranial volume	0.341	0.074	0.393	0.062

	Full correlation estimate (Kendall's tau)	Full correlation p value	Partial correlation estimate (Kendall's tau)	Partial correlation p value
20 weeks' gestation fT4 (N=40)				
Total grey matter volume (N=39)	-0.121	0.281	-0.156	0.180
Cortical volume (N=39)	-0.091	0.417	-0.132	0.258
Cerebral white matter volume	-0.134	0.225	-0.128	0.266
Subcortical grey matter volume	-0.170	0.124	-0.187	0.104
Total intracranial volume	-0.167	0.130	-0.139	0.224
Optimally-treated patients only (N=20)				
Total grey matter volume	-0.159	0.330	-0.203	0.255
Cortical volume	-0.180	0.269	-0.221	0.217
Cerebral white matter volume	0.000	1.000	-0.070	0.695
Subcortical grey matter volume	-0.063	0.697	-0.131	0.462
Total intracranial volume	-0.021	0.897	-0.095	0.595
Over-treated patients only (N=19)				
Total grey matter volume (N=17)	-0.245	0.173	-0.292	0.146
Cortical volume (N=17)	-0.186	0.302	-0.216	0.282
Cerebral white matter volume	-0.248	0.141	-0.243	0.189
Subcortical grey matter volume	-0.260	0.123	-0.303	0.101
Total intracranial volume	-0.271	0.107	-0.278	0.133

	Full correlation estimate (Kendall's tau)	Full correlation p value	Partial correlation estimate (Kendall's tau)	Partial correlation p value
30 weeks' gestation TSH (N=33)				
Total grey matter volume	0.137	0.278	0.123	0.339
Cortical volume (N=31)	0.174	0.182	0.157	0.240
Cerebral white matter volume	0.177	0.161	0.189	0.143
Subcortical grey matter volume	0.065	0.604	0.059	0.647
Total intracranial volume	0.157	0.214	0.183	0.156
Optimally-treated patients only (N=17)				
Total grey matter volume (N=14)	0.483	0.018†	0.558	**0.017**†
Cortical volume	0.313	0.082	0.317	0.114
Cerebral white matter volume	0.164	0.363	0.216	0.282
Subcortical grey matter volume	0.149	0.408	0.148	0.460
Total intracranial volume	0.149	0.408	0.229	0.254
Over-treated patients only (N=16)				
Total grey matter volume	0.229	0.238	0.259	0.217
Cortical volume	0.174	0.370	0.184	0.380
Cerebral white matter volume	0.321	0.099	0.350	0.096
Subcortical grey matter volume	0.174	0.370	0.188	0.370
Total intracranial volume	0.284	0.143	0.286	0.173

	Full correlation estimate (Kendall's tau)	Full correlation p value	Partial correlation estimate (Kendall's tau)	Partial correlation p value
30 weeks' gestation fT4 (N=38)				
Total grey matter volume	0.039	0.734	-0.009	0.937
Cortical volume	0.039	0.734	-0.014	0.908
Cerebral white matter volume	-0.001	0.990	0.014	0.909
Subcortical grey matter volume	0.087	0.443	0.074	0.533
Total intracranial volume	-0.059	0.606	-0.033	0.783
Optimally-treated patients only (N=20)				
Total grey matter volume	-0.054	0.744	-0.071	0.691
Cortical volume	-0.065	0.695	-0.079	0.657
Cerebral white matter volume	0.000	1.000	0.056	0.753
Subcortical grey matter volume	0.043	0.794	0.078	0.662
Total intracranial volume	0.054	0.744	0.120	0.501
Over-treated patients only (N=18)				
Total grey matter volume	0.026	0.879	0.004	0.983
Cortical volume	0.026	0.879	0.009	0.961
Cerebral white matter volume	-0.026	0.879	-0.033	0.863
Subcortical grey matter volume	0.092	0.596	0.079	0.682
Total intracranial volume	-0.157	0.363	-0.178	0.355

† Effect did not survive correction for multiple comparisons using the Bonferroni method.

Partial correlation models accounted for sex, age and pubertal status at the time of imaging. Statistically significant correlation estimates are presented in **bold**. Note I did not perform corrections for multiple comparisons. Abbreviations: TSH=thyroid stimulating hormone, fT4=free thyroxine.

Table 4.4. Region-specific effects of maternal thyroid stimulating hormone

Region volume	Full Correlation Estimate	Full Correlation P value	Partial Correlation Estimate	Partial Correlation P value
Left hemisphere				
Cortex	0.215	0.005	0.162	**0.037***
Cerebral white matter	0.176	0.018	0.134	0.078
Lateral ventricle	0.019	0.809	-0.010	0.896
Inferior lateral ventricle	-0.014	0.858	-0.049	0.532
Cerebellum white matter	0.098	0.190	0.072	0.342
Cerebellum cortex	0.173	0.021	0.134	0.078
Thalamus	0.103	0.170	0.060	0.437
Caudate	0.196	0.009	0.164	**0.033***
Putamen	0.051	0.504	0.002	0.976
Pallidum	0.046	0.540	0.003	0.973
Hippocampus	0.093	0.215	0.052	0.496
Amygdala	0.097	0.193	0.064	0.399
Accumbens area	0.233	0.002	0.224	**0.003***
Ventral diencephalon	0.217	0.004	0.183	**0.018***
Choroid plexus	0.042	0.578	0.029	0.704
Right hemisphere				
Cortex	0.192	0.011	0.150	0.051
Cerebral white matter	0.186	0.013	0.145	0.057
Lateral ventricle	0.157	0.048	0.146	0.072
Inferior lateral ventricle	0.032	0.671	0.026	0.738
Cerebellum white matter	0.081	0.287	0.065	0.400
Cerebellum cortex	0.186	0.013	0.150	0.049
Thalamus	0.102	0.172	0.053	0.490

Region volume	Full Correlation Estimate	Full Correlation P value	Partial Correlation Estimate	Partial Correlation P value
Caudate	0.202	0.008	0.176	**0.023***
Putamen	0.073	0.332	0.022	0.774
Pallidum	0.160	0.036	0.128	0.101
Hippocampus	0.115	0.127	0.072	0.350
Amygdala	0.131	0.083	0.090	0.242
Accumbens area	0.236	0.002	0.218	**0.004***
Ventral diencephelon	0.259	6.313e-04	0.231	**0.003***
Choroid-plexus	0.016	0.832	-0.017	0.826
White matter hypointensities	0.142	0.067	0.116	0.139
Global and central structures				
Brain (minus ventricles)	0.188	0.013	0.145	0.061
Supra-tentorial(minus ventricles)	0.194	0.010	0.151	0.049
Brain-Stem	0.159	0.033	0.122	0.108
3rd-Ventricle	0.019	0.801	-0.025	0.739
4th-Ventricle	0.079	0.303	0.056	0.470
CSF	0.015	0.848	-0.006	0.934
Optic chiasm	0.081	0.281	0.070	0.362
Corpus callosum				
Posterior	0.124	0.099	0.109	0.157
Mid-posterior	0.194	0.009	0.186	**0.015***
Central	0.083	0.267	0.059	0.441
Mid-anterior	0.091	0.230	0.068	0.381
Anterior	0.092	0.235	0.080	0.316

Partial correlation models were adjusted for child's sex, age and self-rated pubertal status at the time of imaging. Regions with a p value <0.05 highlighted in **bold***.

Region volume	Full Correlation Estimate	Full Correlation P value	Partial Correlation Estimate	Partial Correlation P value

None survived Bonferroni's correction. Abbreviations: TSH=thyroid stimulating hormone; CSF, cerebrospinal fluid.

Table 4.5. Region-specific effects of maternal free T4

Region volume	Full Correlation Estimate	Full Correlation P value	Partial Correlation Estimate	Partial Correlation P value
Left hemisphere				
Cortex	0.015	0.841	-0.039	0.615
Cerebral white matter	0.040	0.591	-0.001	0.988
Lateral ventricle	-0.065	0.401	-0.072	0.357
Inferior lateral ventricle	0.046	0.551	0.025	0.748
Cerebellum white matter	0.004	0.954	-0.014	0.849
Cerebellum cortex	0.015	0.844	-0.023	0.762
Thalamus	0.008	0.918	-0.023	0.765
Caudate	-0.076	0.315	-0.117	0.124
Putamen	-0.049	0.519	-0.091	0.236
Pallidum	0.062	0.415	0.030	0.698
Hippocampus	0.028	0.713	-6.492e-04	0.993
Amygdala	0.079	0.295	0.063	0.408
Accumbens area	0.076	0.309	0.072	0.344
Ventral diencephalon	0.046	0.548	0.011	0.885
Choroid plexus	-0.063	0.402	-0.066	0.385
Right hemisphere				
Cortex	0.026	0.735	-0.031	0.689
Cerebral white matter	0.040	0.591	-0.002	0.981
Lateral ventricle	-0.141	0.070	-0.141	0.073
Inferior lateral ventricle	0.105	0.165	0.110	0.152
Cerebellum white matter	0.036	0.639	0.020	0.798
Cerebellum cortex	0.018	0.808	-0.016	0.830
Thalamus	0.061	0.419	0.019	0.800

Caudate	-0.075	0.329	-0.110	0.155
Putamen	-0.060	0.424	-0.109	0.153
Pallidum	-0.018	0.810	-0.050	0.516
Hippocampus	0.078	0.299	0.056	0.463
Amygdala	0.065	0.393	0.032	0.681
Accumbens area	0.013	0.862	-0.002	0.974
Ventral diencephelon	-0.002	0.978	-0.033	0.663
Choroid plexus	0.028	0.710	0.003	0.970
White matter hypointensities	0.071	0.360	0.046	0.558
Global and central structures				
Brain Volume (minus ventricles)	0.032	0.676	-0.020	0.793
Supra-tentorial (minus ventricles)	0.031	0.679	-0.022	0.769
Brain-Stem	0.017	0.826	-0.018	0.809
3rd-Ventricle	-0.136	0.069	-0.177	**0.019***
4th-Ventricle	0.080	0.294	0.059	0.443
CSF	0.050	0.508	0.036	0.632
Optic chiasm	0.107	0.156	0.096	0.210
Corpus callosum				
Posterior	0.018	0.810	0.007	0.924
Mid-posterior	0.046	0.544	0.040	0.597
Central	0.133	0.077	0.109	0.151
Mid-anterior	0.050	0.511	0.022	0.772
Anterior	-0.005	0.952	-0.016	0.843

Partial correlation models were adjusted for child's sex, age and self-rated pubertal status at the time of imaging. Regions with a p value <0.05 highlighted in **bold***. None survived Bonferroni's correction. Abbreviations: fT4=free thyroxine; CSF, cerebrospinal fluid.

4.5 Discussion

In this prospective follow-up analysis of adolescents from the CATS study, I found no differences in brain morphology among adolescents born to mothers with normal or SGTF, nor an effect of treatment with thyroxine (whether optimally- or over-replaced). There were weak positive associations between maternal TSH at 12 weeks' gestation and total grey matter, cortical and cerebral white matter volumes, but these became insignificant after adjustment for age, sex and pubertal status. In exploratory analysis, several brain region volumes were correlated with TSH at 12 weeks' gestation, even after multivariate adjustment, but none survived correction for multiple comparisons. My findings suggest that any effects of excess maternal thyroid function after 12 weeks' gestation on child neurodevelopment (Hales et al., 2020) are not accompanied by altered brain macrostructural morphology in adolescents. There was a trend of maternal TSH being correlated with some brain regions, but nil conclusive.

In contrast to animal model data, human studies of the effects of altered gestational thyroid function on offspring neurodevelopment are more limited and largely observational in nature (Panagiotou et al., 2022). Some studies have observed an association between mild-to-moderate maternal thyroid dysfunction and several child developmental outcomes, including intelligence, reaction time, verbal development and motor function, particularly when thyroid status is altered in early pregnancy (Haddow et al., 1999, Klein et al., 2001, Li et al., 2010, Ghassabian et al., 2014, Julvez et al., 2013, Finken et al., 2013, Henrichs et al., 2010, Kooistra et al., 2006, Pop et al., 2003). Some of these associations have been significant in several meta-analyses (Thompson et al., 2018, Levie et al., 2018), but interpretation is difficult due to heterogeneity in study design, including differences in ethnicity, iodine status, definitions of hypothyroidism, timing of sampling in pregnancy, and timing of assessment of the childhood neurodevelopmental outcomes.

In contrast with previous, smaller studies measuring IQ, the original CATS study children assessed at age 3 (Lazarus et al., 2012) and re-assessed at age 9 (Hales et

al., 2018) found no effect of maternal SGTF on IQ, nor an effect on cognition of thyroid hormone replacement. Casey *et al*, the other randomised controlled trial of thyroxine supplementation in maternal SGTF, similarly found no benefit on IQ or other neurodevelopmental indices in early childhood (Casey et al., 2017). In contrast to IQ, however, the CATS II study did find an effect of maternal thyroxine supplementation on child behaviour (conduct and hyperactivity) in the CATS cohort at age 9, an observation that was confined to inadvertent hyperthyroidism in the subgroup of mothers who had been over-replaced with thyroxine during pregnancy (Hales et al., 2020). These findings are consistent with observational studies and a meta-analysis which confirmed an association between maternal thyroid dysfunction (both hypo- and hyperthyroidism) and childhood ADHD (Modesto et al., 2015, Andersen et al., 2014). However, the mechanisms in operation in humans are unknown.

Whilst other studies have examined the associations between childhood brain morphology and maternal thyroid status (Ghassabian et al., 2014, Korevaar et al., 2016, Jansen et al., 2019), to date, the CATS III study is the largest study of adolescent brain morphology undertaken predominantly in offspring born to mothers with SGTF, and the CATS study was the first interventional trial to consider the effects of maternal thyroxine supplementation on neurodevelopmental trajectories.

In their sub-analysis of 652 eight-year-old children from the Generation R cohort who underwent MRI assessment, Ghassabian et al. (2014) found no association between maternal hypothyroxinaemia in early gestation and brain volume, cortical thickness or surface area despite a 4.3 point reduction in IQ. However, the definition of maternal hypothyroxinaemia was broad, defined as the lowest 5% rather than 2.5% of the cohort's fT4 results, a total of 27 cases. Korevaar et al. (2016) later demonstrated an inverse U-shaped association between maternal free T4 concentrations and child IQ, child grey matter volume and cortical volume in this cohort, a relationship which persisted even after exclusion of mothers with overt thyroid disease. The relationship with TSH lost statistical significance after correction for total intracranial volume; however, intracranial volume and total grey matter volume are strongly linked, hence this may be an unnecessary over-correction. When the same data were later re-analysed by Jansen et al. (2019), excluding

participants in whom only one of fT4 or TSH was available, the relationship of grey matter volume with TSH but not fT4 became significant.

The CATS III results found a weak positive association between maternal TSH at 12 weeks' gestation and child grey matter volume, using a similar methodological imaging approach (3T MRI and automated segmentation by Freesurfer image analysis), but statistical significance was lost after adjustment for potential confounders. The lack of statistically significant differences in CATS III compared to the Generation R studies, despite a greater number of participants exposed to maternal thyroid dysfunction in our study, may be attributable at least in part to the smaller sample size in our study. Other potential explanations may lie in the older adolescent population in CATS III needing adjustment for the potential confounding influences of puberty and its effects on grey matter maturation (Bramen et al., 2011) and the later sampling time of maternal TSH compared to Generation R cohort. Jansen et al. (2019) found that the inverse U-shaped association of maternal TSH with grey matter volume in their study was only evident in early gestation, disappearing entirely after 14 weeks (Jansen et al., 2019). A more significant effect of maternal thyroid status on child brain volume may therefore have been missed in the CATS III study due to the slightly later thyroid sampling. The statistical approach used in this chapter also differed by design, as most participants in the CATS III study were from mothers with SGTF and TSH values were therefore skewed. Furthermore, there was no trend in the raw data that suggested that a non-linear analysis was more appropriate than a linear model, hence I did not undertake cubic spline analysis to examine for non-linear associations.

No evidence in this study was found of an effect of maternal supplementation with levothyroxine on offspring brain macrostructure, even in over-treated mothers in whom offspring conduct and hyperactivity scores above clinically relevant thresholds were more prevalent at age 9 (Hales et al., 2020). This may reflect a lack of power due to the smaller sample size or could be that any neurobehavioral differences are not accompanied by significant changes in brain macrostructure. Alternatively, it is possible that any effects of maternal thyroid function on neurodevelopment are transient: ADHD is often considered as a disorder of delayed brain maturation (Hoogman et al., 2017) associated with amelioration of the phenotype with age (Thapar and Cooper, 2016).

In a previous study of over 18,000 image-derived phenotypes in the UK, a recorded diagnosis of hypothyroidism in adults was associated with a reduction in cerebellar and pallidum grey matter volume (Chambers et al., 2021). Additionally, polygenic risk scores for thyroid traits in the Biobank study demonstrated opposing patterns of association between hypo- and hyperthyroidism at level of pallidum. Recognising that an effect of maternal thyroid status might thus only be apparent at a regional brain level, I undertook additional analyses to examine for associations with subcortical volumes. As with the more global measures, weak positive correlations of baseline maternal TSH with several brain regions were identified, although these are presented uncorrected for possible type 1 error and are therefore purely exploratory in nature.

Other subcortical structures were identified as having weak to modest correlations with TSH, and survived adjustment for puberty, sex and age. However, this was done as part of exploratory analysis and are presented for information only, as I did not adjust for multiple testing, hence there needs to be some caution in interpreting these data. The regions identified were the nucleus accumbens, caudate nuclei and ventral diencephalon in both hemispheres, and mid-posterior corpus callosum (CC). For fT4, only the third ventricle was found to have a statistically significant correlation after adjustments for variables.

The nucleus accumbens (NAcc) in both hemispheres both had a weak to moderate positive correlation with TSH. The NAcc are part of the basal forebrain and are a motor-limbic interface as part of the mesolimbic dopamine pathway. It is involved in the cognitive processing of motor function in relation to reward and motivation pathways (Volkow et al., 2011). Damage to the NAcc has been linked to reduced motivation/ ambition, reduced social behaviour and could contribute to addictive traits (Yael et al., 2019). Disruption in motivation traits have been found in children and adults with ADHD. In both human and animal studies, there are correlations with reduced NAcc volumes and increased ADHD traits (Volkow et al., 2011).

The ventral diencephalon is part of the forebrain and consists of several structures either side of the third ventricle, including the thalamus and hypothalamus (ten Donkelaar et al., 2015). The thalamus is known to have a modulatory role in both afferent and efferent signalling between the cortex and subcortical structures. The

thalamus is the source of waking-state EEG rhythms, and is key in regulating consciousness, arousal and volition (Bailey and Joyce, 2015). A common component of the diagnostic criteria for ADHD are a disturbance in sleep modulation and daytime under arousal (Stephens et al., 2013).

Both caudate nuclei had a statistically significant correlation with maternal TSH at 12 weeks' gestation. Even after adjusting for variables, but strength of the correlation was weak. It is a subcortical structure that forms part of the basal ganglia. It receives afferent projections from almost all regions of the cerebral cortex, with extensive connections to the frontal lobes. The caudate nucleus is, important in coordinating movement and several aspects of cognition and emotion. The caudate nucleus is involved in various cognitive functions, including attention, memory, and learning, as well as emotional regulation (Driscoll, Bollu and Tadi, 2024). Clinically, smaller caudate volumes have been linked to several neurological and psychiatric conditions. For instance, reduced caudate size is associated with lower intelligence quotient (IQ) in children born preterm and impairments in cognitive control and verbal learning/recall in youths exposed to alcohol in utero (Kennedy et al., 2022). Structural and functional MRI abnormalities have also been observed in studies of obsessive compulsive disorder (OCD), Parkinson disease and ADHD (Dang et al., 2016; Driscoll, Bollu and Tadi, 2024). The significance of this weak correlation is unclear, especially as none of the other basal ganglia structures appeared associated with maternal TSH or fT4, but could be an area of interest. In the small number of brain scans of people with cretinism, there was a high proportion with basal ganglia calcification on CT scan (Halpern et al., 1991) and MRI changes in the basal ganglia in a case series of three (Ma et al., 1993).

The corpus callosum is divided into seven regions by FreeSurfer software, as illustrated in Figure 4.3. Only the mid-posterior region had a significant p-value for a weak to moderate correlation with maternal TSH at 12 weeks'. In a previous neuroimaging study looking at effects of maternal hypothyroidism during gestation, the posterior midbody part of the corpus callosum was also larger in those exposed compared to controls (Samadi et al., 2015). Some differences in the corpus callosum have been found in DTI studies, again in the more posterior sections such as isthmus and splenium (Hung et al, 2018; Ng et al., 2014; Copper et al., 2019), but evidence is still limited.

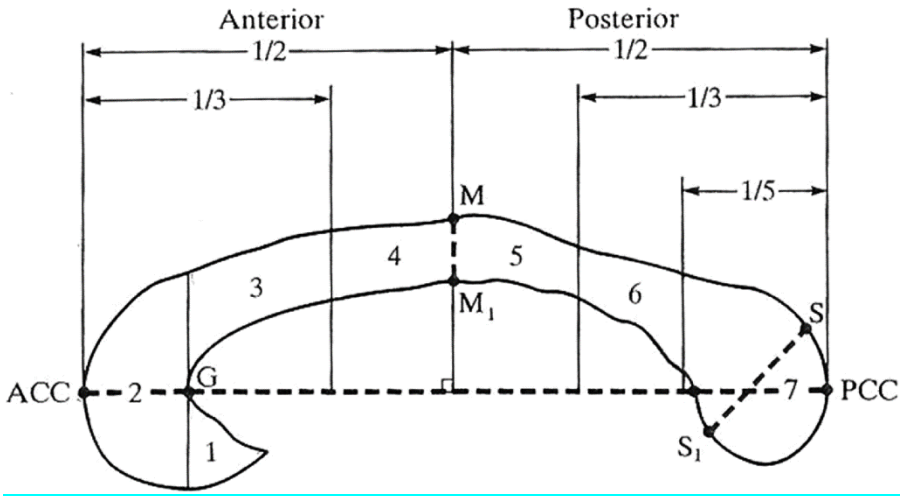


Figure 4.3. Regional subdivisions of the corpus callosum Subregions: (1) rostrum, (2) genu, (3) rostral body, (4) anterior midbody, (5) posterior midbody, (6) isthmus, (7) splenium. Figure taken from Tanaka-Arakawa et al. (2015).

Strengths and limitations

The CATS III study has a number of strengths, including a unique study population from the first RCT examining effect of levothyroxine supplementation during gestation, that also included a subgroup inadvertently exposed to excess thyroid hormones. Other strengths include the increased signal-to-noise ratio offered by 3T MRI and being careful to adjust for the potential confounding influences of puberty as well as age and sex on brain maturation. However, the study also has several limitations, include the lack of repeat neurobehavioral assessment and an evaluation of pubertal status by self-assessment rather than clinician rating. The self-assessment of pubertal status approach is nevertheless valid (Taylor et al., 2001). The participants were predominantly white, reflecting the population of Wales during recruitment, as recorded by the 2011 UK census, but potentially limits the generalisability of the findings to other populations. Another limitation is that participants were each scanned only once, so unable to offer insight into any potential effect of maternal thyroid status on the trajectory of brain development. Although groups were well matched for age, the age range was wider than desired, as adolescence is a time of significant developmental change. Hopefully using pubertal status as a variable will have helped compensate for this. Finally, the sample size is relatively small. A sensitivity analysis performed in R using the pwr package (https://cran.r-project.org/web/packages/pwr/vignettes/pwr-vignette.html) shows the study would have 80% power to detect a medium to large effect size

based on our sample of 20 participants per group. Sample size was limited by the size of the over-treated group in the CATS II study. I invited all 40 subjects from CATS II in the overtreated group, of whom 20 agreed to participate in CATS III. Despite the small group sizes, the CATS III study includes the largest collection, to date, of MRI imaging of children exposed in utero to maternal suboptimal gestational thyroid function (61 SGTF: 24 controls).

4.6 Conclusion

In conclusion, I found no evidence of a significant effect of treating mild maternal thyroid dysfunction on adolescent brain morphology in this follow-up of participants from the Controlled Antenatal Thyroid Screening study. Secondary analysis found maternal TSH in the first trimester to have a weak correlation with several grey matter regional volumes, which also have been implicated in other imaging studies with ADHD. They survived adjustment for variables, but none survived correction for multiple testing. Further studies are needed to investigate the mechanisms by which gestational thyroid function affects human brain development. Other MRI modalities, such as diffusion tensor imaging, or serial imaging may offer a more comprehensive evaluation of the influences of gestational thyroid function on neurodevelopment.

Chapter 5

Chapter 5. Conclusions

5.1 Introduction

In this thesis I have investigated the hypothesis that abnormal thyroid hormone bioavailability in gestation alters brain morphology. I conducted a systematic review of the current MRI neuroimaging literature concerning exposure to abnormal thyroid function during childhood or gestation, and analysed the MRI data from the CATS III study, looking at the effect of suboptimal maternal thyroid dysfunction and effect of levothyroxine replacement during gestation. The current published literature concerning mild suboptimal gestational thyroid function is limited, with conflicting results of whether any significant differences in brain macrostructure are found. Those exposed to a greater degree of maternal thyroid hormone deficiency had more significant results, and overall the earlier the thyroid abnormalities occurred in gestation, the greater the effect on brain morphology measures. The CATS III T1-weighted results found only weak to moderate correlations to maternal TSH at 12 weeks' gestation in regards to grey matter volumes in secondary and exploratory analyses. The intervention with levothyroxine supplementation did not lead to significant differences between the four groups included in the study. The issue with structural MRI studies is the issue of multiple testing due to the large amount of results generated, which impacts on overall confidence in the statistical significance of potentially small differences. It may be that the effect of SGTF is at a more micro- rather than macrostructural level within the brain, for which T1-weighted MRI is not the best modality.

It is irrefutable that neurological cretinism, the most severe form of maternal thyroid dysfunction in pregnancy, has permanent and serious life-long effects on offspring, including significant growth, motor, hearing and cognitive abnormalities. However, it remains unclear about the underlying mechanisms of how and where in the brain this deficiency of available fT4 during the first half of gestation impacts neurodevelopment. Extremely few neuroimaging studies or reports have been published regarding people with cretinism (Halpern et al., 1991; Ma et al ., 1993), as thankfully, with iodine supplementation, it is now very rare. There is a growing body of evidence that less severe thyroid abnormalities during gestation or early life can impact offspring's future neurodevelopment, with an increasing interest in traits of ADHD and autism spectrum disorders. Again, mechanisms of potential action are

still unknown. It continues to be hotly debated in clinical circles, especially whether or not universal antenatal screening of maternal thyroid function should be implemented. The effect of over replacement of T4 is less well known; a small but increasing body of evidence suggests even mild to moderate excess of thyroid hormones during gestation and early childhood is not benign.

5.2 Summary of main findings

Systematic review

I have conducted the first systematic review to date of the effects of various types of thyroid dysfunction on MRI neuroimaging results. I intentionally designed the search criteria to be comprehensive, to allow capture of all relevant studies relating to effect of thyroid hormone abnormalities on the developing brain in childhood, not only during gestation. The included studies consisted of three different types of exposure to thyroid dysfunction: maternal thyroid dysfunction (MTDF), congenital hypothyroidism (CH), and transient hypothyroxinaemia of prematurity (THOP). No neuroimaging studies meeting the inclusion criteria were found for other types of thyroid dysfunction during childhood, e.g. non congenital hypothyroidism causes of hypothyroidism (e.g. autoimmune), childhood hyperthyroidism or genetic causes (e.g. thyroid hormone resistance). All included studies were in neonates of children, with no participants followed up to adulthood.

The included studies were heterogeneous in their design and regions of the brain studied. Those areas that did overlap had conflicting results: some showed associations between neuroimaging findings and TSH levels, while others found stronger correlations with fT4 levels. Notably, in studies with both TSH and fT4 data, often only one hormone showed significant associations with imaging findings, contrary to expectations of reciprocal relationship.

The included MTDF studies did provide evidence that in the first trimester the foetal brain is particularly sensitive to thyroid abnormalities (Lischinsky et al., 2016; Jansen et al., 2019); this finding fits with the developmental timeline of the foetal thyroid gland beginning to produce thyroid hormones of its own during the second half of pregnancy. It also highlights the difficulty with meaningful clinical intervention in early pregnancy to identify and treat asymptomatic mothers with abnormal thyroid function. Unexpectedly, Korevaar et al., (2016) and Jansen et al. (2019) found a

similar effect of fT4 at upper end of normal range and TSH at lower normal range (i.e. borderline subclinical hyperthyroidism) on grey matter volumes as that seen with borderline subclinical hypothyroidism or hypothyroxinaemia. This lends more weight to the association of SGTF with neurodevelopmental issues such as ADHD being found at both ends of the thyroid hormone range, not with subclinical hypothyroidism alone (Andersen et al., 2014 and 2018; Thompson et al., 2018 and MD thesis).

I found several of the CH and THOP neuroimaging studies also reported macrostructural and microstructural brain differences compared to controls, indicating that thyroid hormone deficiencies later in gestation or during the neonatal period can also have long-term impacts on brain morphology. These findings fit with previous studies of the cognitive and behavioural impacts of these conditions. Despite these observations, the lack of replication and the adjustment for confounders or multiple testing in many studies prevent definitive conclusions.

CATS and T1-weighted MRI

I found no evidence of a significant effect of maternal thyroid dysfunction on adolescent brain morphology compared to adolescents born to euthyroid mothers or effects of levothyroxine treatment in this follow-up of participants from the Controlled Antenatal Thyroid Screening study (Lazarus et al., 2012). Secondary analysis combining the four groups did find maternal TSH in the first trimester to have a weak, statistically significant correlation with several grey matter regional volumes, but none survived correction for multiple testing.

The CATS II study found no difference between IQ measures at age 9 in untreated or treated MTDF or born to euthyroid mothers. This addressed the outstanding question from CATS I that the lack of statistically significant results from the original study may have been that levothyroxine supplementation had been started too late in foetal development. The recognition that a subgroup of the treated group were inadvertently over-replaced with levothyroxine led to the secondary analysis in CATS II of an overtreated and optimally treated group. The over-treated group had increased traits of ADHD as reported by parental questionnaires (Hales et al., 2020). This led to the addition of over-replaced as a fourth group in the CATS III study design.

The CATS III study is the first neuroimaging study to date to include a group of children known to have been exposed to a maternal hyperthyroid state *in utero* after the first trimester. Only two of the Generation R imaging studies included specific consideration of effect of higher fT4 or lower TSH on brain morphology (Korevaar et al, 2016; Jansen et al., 2019), but these were all still within normal ranges of thyroid function. The over-replaced group was limited in size by the nature of the CATS II cohort, with only ~40 subjects available to be invited to take part, with an approximately half agreeing to taking part in CATS III. Therefore, power was an issue, especially when assessing the effect of treatment at weeks 20 and 30 or gestation, as results only available for those whose mothers received levothyroxine replacement.

5.2.1 Strengths and limitations

This thesis has several key strengths. A broad and thorough systematic review ensured comprehensive coverage of studies on thyroid hormone abnormalities and their impact on the developing brain, minimizing the risk of missing significant studies. The quality of the included studies was generally high, despite some heterogeneity in the brain regions examined and thyroid hormones measured. The CATS III study also contributed significantly, using 3T MRI technology, a unique, prospective cohort including a group exposed to a mildly hyperthyroid state, well matched groups and analyses adjusted for confounding factors like puberty, age, and sex, as well as multiple testing.

However, the thesis also has limitations. The total number of participants in the included studies in chapter 3 initially appeared substantial. However, when divided among the different thyroid abnormalities, and taking into account that the same participant scan data was often used in two or more of the studies, the sample sizes for each became relatively small. Disparities in the specific thyroid hormones measured (TSH, fT4, or both) and variations in study designs prevented a meta-analysis. The majority of studies focused on thyroid hormone deficiency rather than excess, though a few indicated potential neurodevelopmental impacts from high-normal thyroid hormone levels or levothyroxine supplementation (Jansen et al., 2019; Scratch et al., 2014).

The predominance of 1.5T MRI scans in the reviewed studies, compared to higher resolution scanners available today, may have limited the detection of structural brain differences. Neurological or untreated myxoedematous (CH) cretinism is now rare, and the handful of cases with published imaging data in the literature have been in rural populations (Halpern et al., 1991; Ma et al., 1993; Gupta et al., 1995; Jagannathan et al., 1998), in countries with areas of significant poverty, presumably with the families affected having poor access to adequate nutrition or medical care. The use of MRI for clinical or research use is a specialised and expensive resource, further limiting access to populations most at risk of cretinism.

The CATS III study faced limitations such as group size and the lack of repeated neurobehavioral assessments. The homogeneity of the study population (predominantly white, reflecting the Welsh recruitment base) potentially limits the generalisability of findings. Participants were scanned only once, precluding insights into the effects of maternal thyroid status on brain development trajectories. The relatively small sample size, especially in the over-treated group, posed power limitations, although the study still represents the largest cohort of children exposed to suboptimal gestational thyroid function to date with MRI imaging data (66/85 participants).

5.2.2 Wider literature and implications for future neuroimaging studies

The findings from the CATS III study, although mostly negative, still has suggestions that thyroid function may have some weak correlations with grey matter volumes in certain brain regions. When added to the growing body of evidence that maternal thyroid function during pregnancy significantly influences offspring neurodevelopment and similar trends in other neuroimaging studies, there is an argument for further studies using other MRI techniques.

Secondary analyses suggest that TSH, rather than maternal fT4, is the most sensitive marker of thyroid status. The mild degree of MTDF in CATS III and the Generation R studies, with no IQ differences in CATS participants, aligns with the lack of significant morphological differences. However, differences in ADHD traits in CATS II, alongside consistent findings of neurodevelopmental differences in children exposed to subclinical gestational thyroid dysfunction (SGTF), indicate underlying

brain development differences. This underscores the need for further research to elucidate specific mechanisms and outcomes.

T1-weighted structural MRI has been the primary modality used to investigate MTDF, giving useful insight into macrostructural brain morphology. The main data reported has been on grey matter volumes and anatomically well-defined white matter structures such as the corpus callosum. With increasing interest in white matter microstructure and increasing availability of DTI as a clinical research tool, the CATS III MRI scanning protocol included DTI and qMT modalities as well as T1-weighted MRI. DTI offers a promising avenue to explore white matter integrity and connectivity (Winklewski et al., 2018; Grover et al., 2015). DTI metrics can indicate myelin integrity but may be influenced by other microstructural changes. Including quantitative magnetisation transfer imaging (qMT) to the CATS III scan protocol is unique and provides a direct assessment of myelin, helping to determine if DTI changes relate to myelination (van der Weijden et al., 2023) No DTI studies of MTDF were included in Chapter 3, making the next stage of CATS III study an exciting opportunity to be the first to investigate white matter microstructure in MTDF.

To effectively analyse DTI and qMT data, it is important to focus on key areas of interest to minimise the issue with multiple testing. In the next stage of CATS III, the analysis plans of which white matter tracts to analyse will be based on tracts with clinical and/or neuroimaging evidence suggesting involvement in thyroid-related neurodevelopmental outcomes. Previous animal and human studies have highlighted critical brain regions affected by thyroid hormones. For instance, MCT8 deficiency shows significant myelination abnormalities and MRS results similar to untreated congenital hypothyroidism (Gupta et al., 1995; Jagannathan et al., 1998; Groenweg et al., 2020), suggesting a global delay in myelination that improves over time, although the disease phenotype remains unchanged. A recent murine study of TRIAC, an experimental thyroid hormone analogue, shows potential therapeutic benefits in cortical myelination and neuronal development (Reinweld et al., 2022), and case reports of improved clinical symptoms in human subjects (van Geest et al., 2021).

CT and MRI studies of neurological cretinism report increased basal ganglia abnormalities, with common extrapyramidal motor disorders not directly correlated

with motor abnormalities. Motor issues are also seen in CH but may be less significant in MTDF (Rovet, 1999). The THOP study could provide further insights. Sensorineural hearing loss, common in iodine-deficient areas before supplementation (Gosling et al., 1975) and in children with CH (Cooper et al., 2019), raises questions about the broader impact of thyroid dysfunction on brain development, suggesting hearing loss could be a confounding factor (Armstrong et al., 2020). The extent of hearing impairment in MTDF, whether due to hypothyroidism, subclinical hypothyroidism, or hypothyroxinaemia, remains unclear and warrants further study.

The impact of thyroid dysfunction on myelination and white matter development highlights the future utility of DTI in this research area. The association between SGTF, at either end of the normal spectrum of maternal thyroid function, and ADHD in offspring (Modesto et al., 2015; Andersen et al., 2014; Fetene et al., 2017) could help guide white matter tracts to focus on, given the extensive neuroimaging research in ADHD.

To effectively analyse the DTI data in the upcoming phase of the CATS III study, it is crucial to identify and focus on key regions of interest (ROIs) that have been implicated in thyroid-related neurodevelopmental outcomes and/ or ADHD. Thyroid function studies highlight the importance of regions such as the corpus callosum, hippocampus, thalami, and cerebellum (Morreale de Escobar, 2004; Zendel et al., 2013; Clairman et al., 2015; Navarro et al., 2015; Bernal 2018; Cooper et al., 2019). ADHD has been associated with deficits in white matter integrity across several brain regions, including the cingulum bundle (Amico et al., 2011), corpus callosum, inferior and superior longitudinal fasciculus (Makris et al., 2008), internal capsule, middle cerebellar peduncle, and corticospinal tract (Hyde et al. 2021). By focusing on these regions and tracts, we aim to uncover the specific ways in which maternal thyroid function influences offspring white matter integrity and connectivity. This targeted approach will provide valuable insights into the neurodevelopmental pathways affected by thyroid dysfunction, potentially informing early intervention strategies and improving neurodevelopmental outcomes for children at risk.

5.2.3 Wider literature and implications for effect of gestational thyroid function

In the CATS III study, no significant differences were detected between the four groups, including the group receiving over-replacement of levothyroxine. The CATS III study did not repeat the parental questionnaires, therefore it remains unknown if ADHD traits at age 9 were still present in adolescence. Symptoms of ADHD often reduce over the course of childhood (de Rossi et al., 2023), and there is ongoing debate on definitions and investigations of ADHD (Drechsler et al., 2020). Whilst I cannot conclusively determine the long-term impact on ADHD traits in the CATS cohort, the lack of significant adverse effects on brain macrostructure from the inadvertent over-replacement of levothyroxine is a reassuring outcome.

There are indications that both excess thyroid function and thyroid hormone deficiency can impact child development. Investigating the effects of high fT4 and low TSH on neuroimaging findings remains of significant interest. Elucidating potential negative effects of over-replacing thyroxine is especially important in the wider debate about whether to offer universal screening of maternal thyroid function in pregnancy.

Subclinical hypothyroidism (SCH) has been of interest in obstetric outcome studies as well as in studies investigating longer term neurodevelopmental effects. Several meta-analyses of observational studies have found an association between SCH and several adverse obstetric outcomes, such as pre-eclampsia (Toloza et al., 2022), small for gestational age at birth (Derakhshan et al., 2020), preterm birth (Korevaar et al., 2019) and pregnancy loss (Liu et al., 2014). See Table 5.1 for types and prevalence in pregnancy of different maternal thyroid function abnormalities (Chan et al, 2023). Effect of intervention with levothyroxine has been variable, but appears to be of benefit if started early, well within the first trimester. The effect of isolated hypothyroxinaemia is unclear.

There is ongoing debate on whether there should be universal of thyroid function during early antenatal care, or only targeted screening of women with risk factors of thyroid disease, e.g. age, family history, other autoimmune diseases (Vaidya et al., 2007; Stagnaro-Green et al, 2020). National guidelines exist in several countries, but approaches vary significantly (Stagnaro-Green et al., 2020). There is clear evidence

of overt hypothyroidism being bad for both obstetric and neurodevelopmental outcomes. Although prevalence of underdiagnosed overt hypothyroidism in pregnancy is low, the test and treatment are safe, simple and affordable, but overall benefit and cost effectiveness of universal screening is debated. Concern regarding universal screening antenatally is that there will be many more women who are found to have much more borderline, subclinical thyroid hormone abnormalities, the clinical importance of intervening with remaining unclear, and risks overdiagnosis, associated concern to the mother and potential unneeded treatment leading to iatrogenic harm. The concerns balancing potential gains with potential adverse effects of overtreatment with levothyroxine is well illustrated in Figure 5.1 (Korevaar et al., 2017).

Table 5.1: Common thyroid function disorders in pregnancy and prevalence (Chan et al., 2023)

Condition	**Definition by thyroid function testing**		**Reported prevalence in pregnancies**[a]
	TSH concentration	**fT4 concentration**	
Overt hypothyroidism (OH)	Increased	Decreased	0.2–1% (including undiagnosed, partially-treated and adequately-treated hypothyroidism)
Subclinical hypothyroidism (SCH)	Increased	Normal	2.2–10%
Isolated hypothyroxinaemia (IH)	Normal	Decreased	1.3–8%
Thyrotoxicosis[b] **(including gestational transient thyrotoxicosis)**	Decreased	Increased	1–5%
Overt hyperthyroidism[b] **(Graves' disease and toxic nodular hyperthyroidism)**	Decreased	Increased	0.05–1.3%
Subclinical hyperthyroidism	Decreased	Normal	1.5–2.0%

[a]Using trimester-specific reference ranges in iodine replete and mild-moderately iodine deficient populations. Excludes populations with severe iodine deficiency.

[b]Hyperthyroidism is the increased production and secretion of thyroid hormones whereas thyrotoxicosis refers to the clinical symptoms and signs of excess circulating thyroid hormones, which may not be due to excess 158 thyroid hormone production or hyperthyroidism. Hence, hyperthyroidism comprises a subset of thyrotoxic cases.

Abbreviations: TSH, thyroid stimulating hormone; fT4, free thyroxine

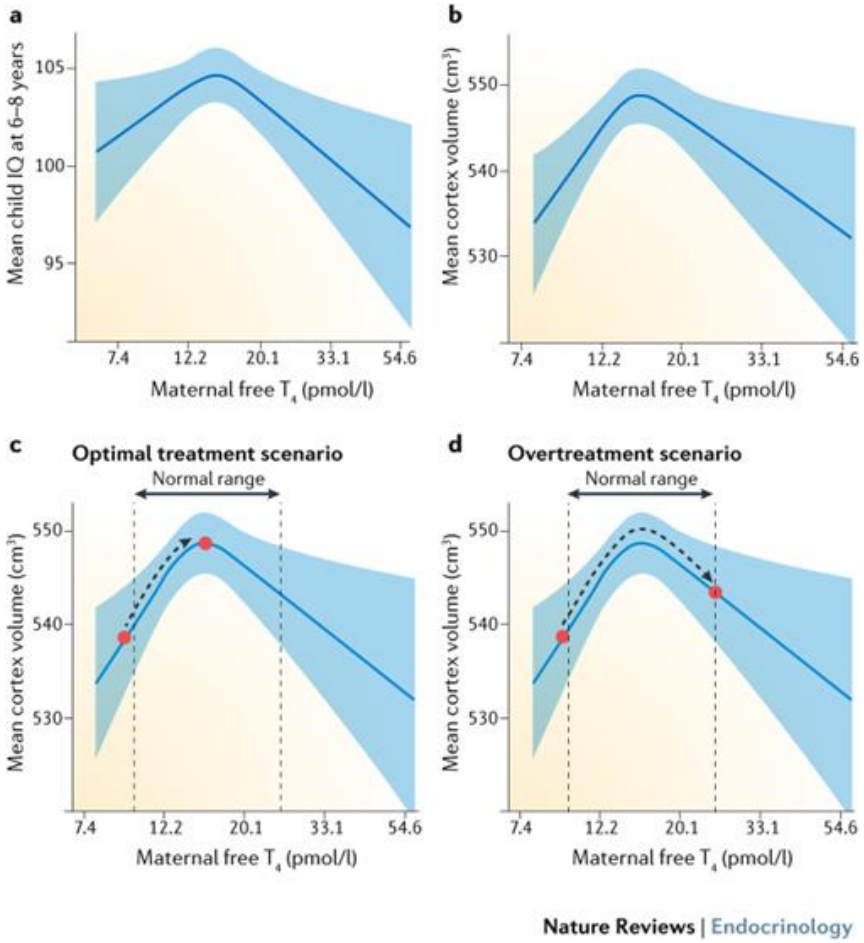


Figure 5.1 Association between maternal free T4 concentrations during early pregnancy and child IQ and cortical volume as well as postulated treatment strategies. Taken from Korevaar et al., 2017. Graphs showing the association between maternal free T4 concentrations during early pregnancy (8–18 weeks) and child IQ at median age of 6 years (a) or mean cortical volume, as assessed by magnetic resonance imaging, at median age of 8 years (b). The lower two graphs show the optimal treatment scenario (c) and the overtreatment scenario (d). These two graphs depict the concept of overtreatment. The red dots represent the free T4 concentrations of a hypothetical patient before (left red dot) and after treatment (right red dot). The vertical dotted black lines display the upper and lower limit of the population-based, assay-specific free T4 reference range for that particular study. Reprinted from The Lancet Diabetes & Endocrinology, 4, Korevaar, Tim I. M. et al. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study, 35–43, Copyright (2016), with permission from Elsevier.

The current draft Royal College of Obstericians and Gynaecologists (RCOG) guidance due to be published later this year appear to be taking a balanced and cautious approach (Chan et al., 2023). The draft recommendation is for targeted screening of women deemed at risk of thyroid disease, using both TSH and fT4 to prevent delays in intervention. The focus will be on TSH rather than fT4. For pregnant women with a TSH of 4 mU/l or above, levothyroxine will be recommended to be started. The aim for those already diagnosed with overt hypothyroidism and on levothyroxine treatment, is to have TSH kept in the lower half of usual normal range, between 0.1-2.5 mU/l, during pregnancy (Chan et al., 2023). Pre-conception optimisation of treatment of known hypothyroidism remains a key aim, although often not achieved in a substantial minority of patients (Okosieme et al., 2018).

The draft RCOG guidelines also advise for further research in this area, including potential effects of higher levels of thyroid hormones on obstetric, foetal and longer-term outcomes. While studying hyperthyroidism is more challenging due to its relative rarity compared to hypothyroidism, future research could focus on recruiting women of childbearing age who are treated for thyroid cancer and intentionally over-treated with levothyroxine to suppress TSH.

5.3 Conclusion

Cretinism and measures of neurodevelopment studies and animal models make a strong argument for the effect of thyroid function in gestation and early childhood on future neurodevelopment. However, type of effect, mechanisms and magnitude/ clinical significance of less severe maternal thyroid dysfunction on offspring central nervous system deveopment is still under debate. It is especially pertinent in the increasing shift towards universal screening for maternal thyroid dysfunction pre-conception or antenatally (Stagnaro-Green et al., 2020). The upcoming RCOG draft guidelines strike a balance, focusing on hypothyroidism rather than hypothyroxinaemia (Chan et al., 2023). This is especially important in the small but growing body of evidence that exposure *in utero* to even mildly elevated/ upper end of normal range of fT4 may not benign.

In conclusion, integrating advanced neuroimaging techniques like DTI in the CATS III study represents a significant step forward in understanding the neurodevelopmental impact of maternal thyroid function. This research will contribute to a more nuanced

understanding of the mechanisms underlying ADHD and other cognitive deficits associated with thyroid dysfunction, ultimately guiding clinical practices and public health policies towards better maternal and child health outcomes.

Chapter 6

Bibliography

6.1 Bibliography

ADAMO, A. M., ALOISE, P. A., SOTO, E. F. & PASQUINI, J. M. 1990. Neonatal hyperthyroidism in the rat produces an increase in the activity of microperoxisomal marker enzymes coincident with biochemical signs of accelerated myelination. J Neurosci Res, 25, 353-9.

AKINCI, A., SARAC, K., GÜNGÖR, S., MUNGAN, I. & AYDIN, O. 2006. Brain MR spectroscopy findings in neonates with hypothyroidism born to mothers living in iodine-deficient areas. AJNR Am J Neuroradiol, 27, 2083-7.

AL SHIDHANI, A., ULLAH, I., ALSAFFAR, H., AL KINDI, A., AL NABHANI, H. & AL YAARUBI, S. 2021. Thyroid Hormone Resistance due to a Novel De Novo Mutation in Thyroid Hormone Receptor Alpha: First Case Report from the Middle East and North Africa. Oman Med J, 36, e226.

ALVAREZ, M., IGLESIAS FERNÁNDEZ, C., RODRÍGUEZ SÁNCHEZ, A., DULÍN LÑIGUEZ, E. & RODRÍGUEZ ARNAO, M. D. 2010. Episodes of overtreatment during the first six months in children with congenital hypothyroidism and their relationships with sustained attention and inhibitory control at school age. Horm Res Paediatr, 74, 114-20.

AMICO, F., STAUBER, J., KOUTSOULERIS, N. & FRODL, T. 2011. Anterior cingulate cortex gray matter abnormalities in adults with attention deficit hyperactivity disorder: a voxel-based morphometry study. Psychiatry Res, 191, 31-5.

ANDERSEN, S. L., ANDERSEN, S., VESTERGAARD, P. & OLSEN, J. 2018. Maternal Thyroid Function in Early Pregnancy and Child Neurodevelopmental Disorders: A Danish Nationwide Case-Cohort Study. Thyroid, 28, 537-546.

ANDERSEN, S. L., LAURBERG, P., WU, C. S. & OLSEN, J. 2014. Attention deficit hyperactivity disorder and autism spectrum disorder in children born to mothers with thyroid dysfunction: a Danish nationwide cohort study. BJOG, 121, 1365-74.

ARMSTRONG, N. M., WILLIAMS, O. A., LANDMAN, B. A., DEAL, J. A., LIN, F. R. & RESNICK, S. M. 2020. Association of Poorer Hearing With Longitudinal Change in

Cerebral White Matter Microstructure. JAMA Otolaryngol Head Neck Surg, 146, 1035-1042.

ARNSTEN, A. F. & RUBIA, K. 2012. Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: disruptions in neurodevelopmental psychiatric disorders. J Am Acad Child Adolesc Psychiatry, 51, 356-67.

ARROJO E DRIGO, R. & BIANCO, A. C. 2011. Type 2 deiodinase at the crossroads of thyroid hormone action. Int J Biochem Cell Biol, 43, 1432-41.

ARROJO E DRIGO, R., FONSECA, T. L., CASTILLO, M., SALATHE, M., SIMOVIC, G., MOHÁCSIK, P., GEREBEN, B. & BIANCO, A. C. 2011. Endoplasmic reticulum stress decreases intracellular thyroid hormone activation via an eIF2a-mediated decrease in type 2 deiodinase synthesis. Mol Endocrinol, 25, 2065-75.

ASSOCIATION, A. P. 2022. Diagnostic and Statistical Manual of Mental Disorders,

Fifth Edition, Text Revision., Arlington, VA, American Psychiatric Association.

AUSÓ, E., LAVADO-AUTRIC, R., CUEVAS, E., DEL REY, F. E., MORREALE DE ESCOBAR, G. & BERBEL, P. 2004. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology, 145, 4037-47.

BAILEY, T. & JOYCE, A. 2015. The role of the thalamus in ADHD symptomatology and treatment. Applied Neuropsychology: Child, 4, 89-96.

BERBEL, P., AUSÓ, E., GARCÍA-VELASCO, J. V., MOLINA, M. L. & CAMACHO, M. 2001. Role of thyroid hormones in the maturation and organisation of rat barrel cortex. Neuroscience, 107, 383-94.

BERBEL, P., GUADAÑO-FERRAZ, A., ANGULO, A. & RAMÓN CEREZO, J. 1994. Role of thyroid hormones in the maturation of interhemispheric connections in rats. Behav Brain Res, 64, 9-14.

BERNAL, J., GUADAÑO-FERRAZ, A. & MORTE, B. 2015. Thyroid hormone transporters-functions and clinical implications. Nat Rev Endocrinol, 11, 690.

BERNAL, J., OBREGON, M. J. & SANTISTEBAN, P. S. 2018. In Memoriam: Gabriella Morreale de Escobar. European Thyroid Journal, 7, 109-110.

BHUTTA, A. T., CLEVES, M. A., CASEY, P. H., CRADOCK, M. M. & ANAND, K. J. 2002. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. JAMA, 288, 728-37.

BIRKEN, D. L. & OLDENDORF, W. H. 1989. N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. Neurosci Biobehav Rev, 13, 23-31.

BLASI, V., LONGARETTI, R., GIOVANETTONI, C., BALDOLI, C., PONTESILLI, S., VIGONE, C., SACCUMAN, C., NIGRO, F., CHIUMELLO, G., SCOTTI, G. & WEBER, G. 2009. Decreased Parietal Cortex Activity during Mental Rotation in Children with Congenital Hypothyroidism. Neuroendocrinology, 89, 56-65.

BLAZER, S., MOREH-WATERMAN, Y., MILLER-LOTAN, R., TAMIR, A. & HOCHBERG, Z. 2003. Maternal hypothyroidism may affect fetal growth and neonatal thyroid function. Obstet Gynecol, 102, 232-41.

BOCHUKOVA, E., SCHOENMAKERS, N., AGOSTINI, M., SCHOENMAKERS, E., RAJANAYAGAM, O., KEOGH, J. M., HENNING, E., REINEMUND, J., GEVERS, E., SARRI, M., DOWNES, K., OFFIAH, A., ALBANESE, A., HALSALL, D., SCHWABE, J. W., BAIN, M., LINDLEY, K., MUNTONI, F., VARGHA-KHADEM, F., DATTANI, M., FAROOQI, I. S., GURNELL, M. & CHATTERJEE, K. 2012. A mutation in the thyroid hormone receptor alpha gene. N Engl J Med, 366, 243-9.

BONGERS-SCHOKKING, J. J., RESING, W. C. M., OOSTDIJK, W., DE RIJKE, Y. B. & DE MUINCK KEIZER-SCHRAMA, S. M. P. F. 2018. Relation between Early Over- and Undertreatment and Behavioural Problems in Preadolescent Children with Congenital Hypothyroidism. Horm Res Paediatr, 90, 247-256.

BOYAGES, S. C., HALPERN, J. P., MABERLY, G. F., EASTMAN, C. J., MORRIS, J., COLLINS, J., JUPP, J. J., JIN, C. E., WANG, Z. H. & YOU, C. Y. 1988. A comparative study of neurological and myxedematous endemic cretinism in western China. J Clin Endocrinol Metab, 67, 1262-71.

BRAMEN, J. E., HRANILOVICH, J. A., DAHL, R. E., FORBES, E. E., CHEN, J., TOGA, A. W., DINOV, I. D., WORTHMAN, C. M. & SOWELL, E. R. 2011. Puberty influences medial temporal lobe and cortical gray matter maturation differently in boys than girls matched for sexual maturity. Cereb Cortex, 21, 636-46.

BROWN, E. D. L., OBENG-GYASI, B., HALL, J. E. & SHEKHAR, S. 2023. The Thyroid Hormone Axis and Female Reproduction. Int J Mol Sci, 24.

BRUCKER-DAVIS, F., SKARULIS, M. C., GRACE, M. B., BENICHOU, J., HAUSER, P., WIGGS, E. & WEINTRAUB, B. D. 1995. Genetic and clinical features of 42 kindreds with resistance to thyroid hormone. The National Institutes of Health Prospective Study. Ann Intern Med, 123, 572-83.

CACI, H., COHEN, D., BONNOT, O., KABUTH, B., RAYNAUD, J. P., PAILLÉ, S. & VALLÉE, L. 2020. Health Care Trajectories for Children With ADHD in France: Results From the QUEST Survey. J Atten Disord, 24, 52-65.

CALVO, R., OBREGÓN, M. J., RUIZ DE OÑA, C., ESCOBAR DEL REY, F. & MORREALE DE ESCOBAR, G. 1990. Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. J Clin Invest, 86, 889-99.

CALZÀ, L., BALDASSARRO, V. A., FERNANDEZ, M., GIULIANI, A., LORENZINI, L. & GIARDINO, L. 2018. Thyroid Hormone and the White Matter of the Central Nervous System: From Development to Repair. Vitam Horm, 106, 253-281.

CAMPBELL, J. S. W., LEPPERT, I. R., NARAYANAN, S., BOUDREAU, M., DUVAL, T., COHEN-ADAD, J., PIKE, G. B. & STIKOV, N. 2018. Promise and pitfalls of g-ratio estimation with MRI. Neuroimage, 182, 80-96.

CAO, X. Y., JIANG, X. M., DOU, Z. H., RAKEMAN, M. A., ZHANG, M. L., O'DONNELL, K., MA, T., AMETTE, K., DELONG, N. & DELONG, G. R. 1994. Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. N Engl J Med, 331, 1739-44.

CASEY, B. M., THOM, E. A., PEACEMAN, A. M., VARNER, M. W., SOROKIN, Y., HIRTZ, D. G., REDDY, U. M., WAPNER, R. J., THORP, J. M., SAADE, G., TITA, A. T., ROUSE, D. J., SIBAI, B., IAMS, J. D., MERCER, B. M., TOLOSA, J., CARITIS, S. N., VANDORSTEN, J. P. & NETWORK, E. K. S. N. I. O. C. H. A. H. D. M. F. M. U. 2017. Treatment of Subclinical Hypothyroidism or Hypothyroxinemia in Pregnancy. N Engl J Med, 376, 815-825.

CHAMBERS, T., ANNEY, R., TAYLOR, P. N., TEUMER, A., PEETERS, R. P., MEDICI, M., CASERAS, X. & REES, D. A. 2021. Effects of Thyroid Status on Regional Brain Volumes: A Diagnostic and Genetic Imaging Study in UK Biobank. J Clin Endocrinol Metab, 106, 688-696.

CHAN, S., KACHILELE, S., MCCABE, C. J., TANNAHILL, L. A., BOELAERT, K., GITTOES, N. J., VISSER, T. J., FRANKLYN, J. A. & KILBY, M. D. 2002. Early expression of thyroid hormone deiodinases and receptors in human fetal cerebral cortex. Brain Res Dev Brain Res, 138, 109-16.

CHAN, S., MARSH, M., BOELAERT, K., EVANS, C., GILBERT, J. & DHILLON-SMITH, R. 2023. RCOG Green-top Guideline (New), May-June 2023 – Peer Review Draft 2023. Management of Thyroid Disorders in Pregnancy. London, UK: Royal College of Obstetricians and Gynaecologists. .

CHEVRIER, J., HARLEY, K. G., KOGUT, K., HOLLAND, N., JOHNSON, C. & ESKENAZI, B. 2011. Maternal Thyroid Function during the Second Half of Pregnancy and Child Neurodevelopment at 6, 12, 24, and 60 Months of Age. J Thyroid Res, 2011, 426427.

CHOUFOER, J. C., VANRHIJN, M. & QUERIDO, A. 1965. ENDEMIC GOITER IN WESTERN NEW GUINEA. II. CLINICAL PICTURE, INCIDENCE AND PATHOGENESIS OF ENDEMIC CRETINISM. J Clin Endocrinol Metab, 25, 385-402.

CLAIRMAN, H., SKOCIC, J., LISCHINSKY, J. E. & ROVET, J. 2015. Do children with congenital hypothyroidism exhibit abnormal cortical morphology? Pediatric Research, 78, 286-297.

COOPER, H. E., KADEN, E., HALLIDAY, L. F., BAMIOU, D. E., MANKAD, K., PETERS, C. & CLARK, C. A. 2019. White matter microstructural abnormalities in children with severe congenital hypothyroidism. Neuroimage Clin, 24, 101980.

CROCKFORD, S. J. 2003. Thyroid rhythm phenotypes and hominid evolution: a new paradigm implicates pulsatile hormone secretion in speciation and adaptation changes. Comp Biochem Physiol A Mol Integr Physiol, 135, 105-29.

CUEVAS, E., AUSÓ, E., TELEFONT, M., MORREALE DE ESCOBAR, G., SOTELO, C. & BERBEL, P. 2005. Transient maternal hypothyroxinemia at onset of

corticogenesis alters tangential migration of medial ganglionic eminence-derived neurons. Eur J Neurosci, 22, 541-51.

CURLING, T. B. 1850. Two Cases of absence of the Thyroid body, and symmetrical swellings of fat tissue at the sides of the neck, connected with defective cerebral development. Med Chir Trans, 33, 303-6.

DAVIE, C. A., HAWKINS, C. P., BARKER, G. J., BRENNAN, A., TOFTS, P. S., MILLER, D. H. & MCDONALD, W. I. 1993. Detection of myelin breakdown products by proton magnetic resonance spectroscopy. Lancet, 341, 630-1.

DAVIS, P. J., GOGLIA, F. & LEONARD, J. L. 2016. Nongenomic actions of thyroid hormone. Nat Rev Endocrinol, 12, 111-21.

DAVIS, P. J., LIN, H. Y., HERCBERGS, A. A., KEATING, K. A. & MOUSA, S. A. 2019. How thyroid hormone works depends upon cell type, receptor type, and hormone analogue: implications in cancer growth. Discov Med, 27, 111-117.

DE CASTRO, G. V., ROCHA, C. M. R., SANCHES, V. L., RIBEIRO, P. H. Z., DONATE, P. M., DE LAURENTIZ, R. D. S., POLIKARPOV, I. & SAIRRE, M. I. 2015. Synthesis of Analogues of Thyroid Hormones: Nuclear Receptor Modulators. Orbital: The Electronic Journal of Chemistry, 7, 282-291.

DE ESCOBAR, G. M., OBREGÓN, M. J. & DEL REY, F. E. 2004. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract Res Clin Endocrinol Metab, 18, 225-48.

DE ROSSI, P., D'AIELLO, B., PRETELLI, I., MENGHINI, D., DI VARA, S. & VICARI, S. 2023. Age-related clinical characteristics of children and adolescents with ADHD. Front Psychiatry, 14, 1069934.

DE ZEGHER, F., PERNASETTI, F., VANHOLE, C., DEVLIEGER, H., VAN DEN BERGHE, G. & MARTIAL, J. A. 1995. The prenatal role of thyroid hormone evidenced by fetomaternal Pit-1 deficiency. J Clin Endocrinol Metab, 80, 3127-30.

DEL RÍO, J. A., HEIMRICH, B., BORRELL, V., FÖRSTER, E., DRAKEW, A., ALCÁNTARA, S., NAKAJIMA, K., MIYATA, T., OGAWA, M., MIKOSHIBA, K., DERER, P., FROTSCHER, M. & SORIANO, E. 1997. A role for Cajal-Retzius cells and reelin in the development of hippocampal connections. Nature, 385, 70-4.

DENVER, R. J., HU, F., SCANLAN, T. S. & FURLOW, J. D. 2009. Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in Xenopus laevis. Dev Biol, 326, 155-68.

DERAKHSHAN, A., PEETERS, R. P., TAYLOR, P. N., BLIDDAL, S., CARTY, D. M., MEEMS, M., VAIDYA, B., CHEN, L., KNIGHT, B. A., GHAFOOR, F., POPOVA, P. V., MOSSO, L., OKEN, E., SUVANTO, E., HISADA, A., YOSHINAGA, J., BROWN, S. J., BASSOLS, J., AUVINEN, J., BRAMER, W. M., LÓPEZ-BERMEJO, A., DAYAN, C. M., FRENCH, R., BOUCAI, L., VAFEIADI, M., GRINEVA, E. N., POP, V. J. M., VRIJKOTTE, T. G., CHATZI, L., SUNYER, J., JIMÉNEZ-ZABALA, A., RIAÑO, I., REBAGLIATO, M., LU, X., PIRZADA, A., MÄNNISTÖ, T., DELLES, C., FELDT-RASMUSSEN, U., ALEXANDER, E. K., NELSON, S. M., CHAKER, L., PEARCE, E. N., GUXENS, M., STEEGERS, E. A. P., WALSH, J. P. & KOREVAAR, T. I. M. 2020. Association of maternal thyroid function with birthweight: a systematic review and individual-participant data meta-analysis. Lancet Diabetes Endocrinol, 8, 501-510.

DESOUZA, L. A., LADIWALA, U., DANIEL, S. M., AGASHE, S., VAIDYA, R. A. & VAIDYA, V. A. 2005. Thyroid hormone regulates hippocampal neurogenesis in the adult rat brain. Mol Cell Neurosci, 29, 414-26.

DESOUZA, L. A., SATHANOORI, M., KAPOOR, R., RAJADHYAKSHA, N., GONZALEZ, L. E., KOTTMANN, A. H., TOLE, S. & VAIDYA, V. A. 2011. Thyroid hormone regulates the expression of the sonic hedgehog signaling pathway in the embryonic and adult Mammalian brain. Endocrinology, 152, 1989-2000.

DOBBING, J. & SANDS, J. 1970. Timing of neuroblast multiplication in developing human brain. Nature, 226, 639-40.

DONKELAAR, H. J. T., WARRENBURG, B. V. D., WILLEMSEN, M., KÜSTERS, B., HASHIZUME, Y. & HORI, A. 2020. Basal ganglia. Clinical Neuroanatomy. Springer.

DRECHSLER, R., BREM, S., BRANDEIS, D., GRÜNBLATT, E., BERGER, G. & WALITZA, S. 2020. ADHD: Current Concepts and Treatments in Children and Adolescents. Neuropediatrics, 51, 315-335.

DRISCOLL ME, B. P., TADI P 2023. StatPearls. Treasure Island (FL): StatPearls Publishing.

DUMONT, J., OPITZ, R., CHRISTOPHE, D., VASSART, G., ROGER, P. P. & MAENHAUT, C. 2011. Ontogeny, anatomy, metabolism and physiology of the thyroid. Thyroid Dis. Manag, 1-102.

ERBAŞ, İ. & DEMIR, K. 2021. The Clinical Spectrum of Resistance to Thyroid Hormone Alpha in Children and Adults. J Clin Res Pediatr Endocrinol, 13, 1-14.

FETENE, D. M., BETTS, K. S. & ALATI, R. 2017. MECHANISMS IN ENDOCRINOLOGY: Maternal thyroid dysfunction during pregnancy and behavioural and psychiatric disorders of children: a systematic review. Eur J Endocrinol, 177, R261-R273.

FINKEN, M. J., VAN EIJSDEN, M., LOOMANS, E. M., VRIJKOTTE, T. G. & ROTTEVEEL, J. 2013. Maternal hypothyroxinemia in early pregnancy predicts reduced performance in reaction time tests in 5- to 6-year-old offspring. J Clin Endocrinol Metab, 98, 1417-26.

FISCHL, B. 2012. FreeSurfer. Neuroimage, 62, 774-81.

FRIEDMAN, L. A. & RAPOPORT, J. L. 2015. Brain development in ADHD. Curr Opin Neurobiol, 30, 106-11.

FRIESEMA, E. C., GRUETERS, A., BIEBERMANN, H., KRUDE, H., VON MOERS, A., REESER, M., BARRETT, T. G., MANCILLA, E. E., SVENSSON, J., KESTER, M. H., KUIPER, G. G., BALKASSMI, S., UITTERLINDEN, A. G., KOEHRLE, J., RODIEN, P., HALESTRAP, A. P. & VISSER, T. J. 2004. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet, 364, 1435-7.

FURLOW, J. D. & NEFF, E. S. 2006. A developmental switch induced by thyroid hormone: Xenopus laevis metamorphosis. Trends Endocrinol Metab, 17, 40-7.

GADIAN, D. 1995. NMR and it Applications to Living Systems. 2nd edn, Oxford, Oxford University Press

GALTON, V. A., DE WAARD, E., PARLOW, A. F., ST GERMAIN, D. L. & HERNANDEZ, A. 2014. Life without the iodothyronine deiodinases. Endocrinology, 155, 4081-7.

GARCÍA-FERNÁNDEZ, L. F., RAUSELL, E., URADE, Y., HAYAISHI, O., BERNAL, J. & MUÑOZ, A. 1997. Hypothyroidism alters the expression of prostaglandin D2 synthase/beta trace in specific areas of the developing rat brain. Eur J Neurosci, 9, 1566-73.

GE, G. M., LEUNG, M. T. Y., MAN, K. K. C., LEUNG, W. C., IP, P., LI, G. H. Y., WONG, I. C. K., KUNG, A. W. C. & CHEUNG, C. L. 2020. Maternal Thyroid Dysfunction During Pregnancy and the Risk of Adverse Outcomes in the Offspring: A Systematic Review and Meta-Analysis. J Clin Endocrinol Metab, 105.

GENC, S., MALPAS, C. B., GULENC, A., SCIBERRAS, E., EFRON, D., SILK, T. J. & SEAL, M. L. 2020. Longitudinal patterns of white matter fibre density and morphology in children are associated with age and pubertal stage. Dev Cogn Neurosci, 45, 100853.

GHASSABIAN, A., BONGERS-SCHOKKING, J. J., DE RIJKE, Y. B., VAN MIL, N., JADDOE, V. W., DE MUINCK KEIZER-SCHRAMA, S. M., HOOIJKAAS, H., HOFMAN, A., VISSER, W., ROMAN, G. C., VISSER, T. J., VERHULST, F. C. & TIEMEIER, H. 2012. Maternal thyroid autoimmunity during pregnancy and the risk of attention deficit/hyperactivity problems in children: the Generation R Study. Thyroid, 22, 178-86.

GHASSABIAN, A., BONGERS-SCHOKKING, J. J., HENRICHS, J., JADDOE, V. W., VISSER, T. J., VISSER, W., DE MUINCK KEIZER-SCHRAMA, S. M., HOOIJKAAS, H., STEEGERS, E. A., HOFMAN, A., VERHULST, F. C., VAN DER ENDE, J., DE RIJKE, Y. B. & TIEMEIER, H. 2011. Maternal thyroid function during pregnancy and behavioral problems in the offspring: the generation R study. Pediatr Res, 69, 454-9.

GHASSABIAN, A., EL MARROUN, H., PEETERS, R. P., JADDOE, V. W., HOFMAN, A., VERHULST, F. C., TIEMEIER, H. & WHITE, T. 2014. Downstream Effects of Maternal Hypothyroxinemia in Early Pregnancy: Nonverbal IQ and Brain Morphology in School-Age Children. Journal of Clinical Endocrinology & Metabolism, 99, 2383-2390.

GOODMAN, R. 1997. The Strengths and Difficulties Questionnaire: a research note. J Child Psychol Psychiatry, 38, 581-6.

GOSLINGS, B. M., DJOKOMOELJANTO, R., HOEDIJONO, R., SOEPARDJO, H. & QUERIDO, A. 1975. Studies on hearing loss in a community with endemic cretinism in Central Java, Indonesia. Acta Endocrinol (Copenh), 78, 705-13.

GROENEWEG, S., VAN GEEST, F. S., ABACI, A., ALCANTUD, A., AMBEGAONKAR, G. P., ARMOUR, C. M., BAKHTIANI, P., BARCA, D., BERTINI, E. S., VAN BEYNUM, I. M., BRUNETTI-PIERRI, N., BUGIANI, M., CAPPA, M., CAPPUCCIO, G., CASTELLOTTI, B., CASTIGLIONI, C., CHATTERJEE, K., DE COO, I. F. M., COUTANT, R., CRAIU, D., CROCK, P., DEGOEDE, C., DEMIR, K., DICA, A., DIMITRI, P., DOLCETTA-CAPUZZO, A., DREMMEN, M. H. G., DUBEY, R., ENDERLI, A., FAIRCHILD, J., GALLICHAN, J., GEORGE, B., GEVERS, E. F., HACKENBERG, A., HALÁSZ, Z., HEINRICH, B., HUYNH, T., KŁOSOWSKA, A., VAN DER KNAAP, M. S., VAN DER KNOOP, M. M., KONRAD, D., KOOLEN, D. A., KRUDE, H., LAWSON-YUEN, A., LEBL, J., LINDER-LUCHT, M., LOREA, C. F., LOURENÇO, C. M., LUNSING, R. J., LYONS, G., MALIKOVA, J., MANCILLA, E. E., MCGOWAN, A., MERICQ, V., LORA, F. M., MORAN, C., MÜLLER, K. E., OLIVER-PETIT, I., PAONE, L., PAUL, P. G., POLAK, M., PORTA, F., POSWAR, F. O., REINAUER, C., ROZENKOVA, K., MENEVSE, T. S., SIMM, P., SIMON, A., SINGH, Y., SPADA, M., VAN DER SPEK, J., STALS, M. A. M., STOUPA, A., SUBRAMANIAN, G. M., TONDUTI, D., TURAN, S., DEN UIL, C. A., VANDERNIET, J., VAN DER WALT, A., WÉMEAU, J. L., WIERZBA, J., DE WIT, M. Y., WOLF, N. I., WURM, M., ZIBORDI, F., ZUNG, A., ZWAVELING-SOONAWALA, N. & VISSER, W. E. 2020. Disease characteristics of MCT8 deficiency: an international, retrospective, multicentre cohort study. Lancet Diabetes Endocrinol, 8, 594-605.

GUPTA, R. K., BHATIA, V., POPTANI, H. & GUJRAL, R. B. 1995. Brain metabolite changes on in vivo proton magnetic resonance spectroscopy in children with congenital hypothyroidism. Journal of Pediatrics, 126, 389-92.

HADDOW, J. E., PALOMAKI, G. E., ALLAN, W. C., WILLIAMS, J. R., KNIGHT, G. J., GAGNON, J., O'HEIR, C. E., MITCHELL, M. L., HERMOS, R. J., WAISBREN, S. E., FAIX, J. D. & KLEIN, R. Z. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med, 341, 549-55.

HALES, C., CHANNON, S., TAYLOR, P. N., DRAMAN, M. S., MULLER, I., LAZARUS, J., PARADICE, R., REES, A., SHILLABEER, D., GREGORY, J. W., DAYAN, C. M. & LUDGATE, M. 2014. The second wave of the Controlled Antenatal Thyroid Screening (CATS II) study: the cognitive assessment protocol. BMC Endocr Disord, 14, 95.

HALES, C., TAYLOR, P. N., CHANNON, S., MCEWAN, K., THAPAR, A., LANGLEY, K., MULLER, I., DRAMAN, M. S., DAYAN, C., GREGORY, J. W., OKOSIEME, O., LAZARUS, J. H., REES, D. A. & LUDGATE, M. 2020. Controlled Antenatal Thyroid Screening II: Effect of Treating Maternal Suboptimal Thyroid Function on Child Behavior. J Clin Endocrinol Metab, 105.

HALES, C., TAYLOR, P. N., CHANNON, S., PARADICE, R., MCEWAN, K., ZHANG, L., GYEDU, M., BAKHSH, A., OKOSIEME, O., MULLER, I., DRAMAN, M. S., GREGORY, J. W., DAYAN, C., LAZARUS, J. H., REES, D. A. & LUDGATE, M. 2018. Controlled Antenatal Thyroid Screening II: Effect of Treating Maternal Suboptimal Thyroid Function on Child Cognition. J Clin Endocrinol Metab, 103, 1583-1591.

HALPERN, J. P., BOYAGES, S. C., MABERLY, G. F., COLLINS, J. K., EASTMAN, C. J. & MORRIS, J. G. 1991. The neurology of endemic cretinism. A study of two endemias. Brain, 114 (Pt 2), 825-41.

HATTEN, M. E. 1990. Riding the glial monorail: a common mechanism for glial-guided neuronal migration in different regions of the developing mammalian brain. Trends Neurosci, 13, 179-84.

HENRICHS, J., BONGERS-SCHOKKING, J. J., SCHENK, J. J., GHASSABIAN, A., SCHMIDT, H. G., VISSER, T. J., HOOIJKAAS, H., DE MUINCK KEIZER-SCHRAMA, S. M., HOFMAN, A., JADDOE, V. V., VISSER, W., STEEGERS, E. A., VERHULST, F. C., DE RIJKE, Y. B. & TIEMEIER, H. 2010. Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: the generation R study. J Clin Endocrinol Metab, 95, 4227-34.

HERCBERGS, A. 2019. Clinical Implications and Impact of Discovery of the Thyroid Hormone Receptor on Integrin αvβ3-A Review. Front Endocrinol (Lausanne), 10, 565.

HERTING, M. M. & SOWELL, E. R. 2017. Puberty and structural brain development in humans. Front Neuroendocrinol, 44, 122-137.

HÖNES, G. S., HÄRTING, N., MITTAG, J. & KAISER, F. J. 2022. TRα2-An Untuned Second Fiddle or Fine-Tuning Thyroid Hormone Action? Int J Mol Sci, 23.

HOOGMAN, M., BRALTEN, J., HIBAR, D. P., MENNES, M., ZWIERS, M. P., SCHWEREN, L. S. J., VAN HULZEN, K. J. E., MEDLAND, S. E., SHUMSKAYA, E., JAHANSHAD, N., ZEEUW, P., SZEKELY, E., SUDRE, G., WOLFERS, T., ONNINK, A. M. H., DAMMERS, J. T., MOSTERT, J. C., VIVES-GILABERT, Y., KOHLS, G., OBERWELLAND, E., SEITZ, J., SCHULTE-RÜTHER, M., AMBROSINO, S., DOYLE, A. E., HØVIK, M. F., DRAMSDAHL, M., TAMM, L., VAN ERP, T. G. M., DALE, A., SCHORK, A., CONZELMANN, A., ZIERHUT, K., BAUR, R., MCCARTHY, H., YONCHEVA, Y. N., CUBILLO, A., CHANTILUKE, K., MEHTA, M. A., PALOYELIS, Y., HOHMANN, S., BAUMEISTER, S., BRAMATI, I., MATTOS, P., TOVAR-MOLL, F., DOUGLAS, P., BANASCHEWSKI, T., BRANDEIS, D., KUNTSI, J., ASHERSON, P., RUBIA, K., KELLY, C., MARTINO, A. D., MILHAM, M. P., CASTELLANOS, F. X., FRODL, T., ZENTIS, M., LESCH, K. P., REIF, A., PAULI, P., JERNIGAN, T. L., HAAVIK, J., PLESSEN, K. J., LUNDERVOLD, A. J., HUGDAHL, K., SEIDMAN, L. J., BIEDERMAN, J., ROMMELSE, N., HESLENFELD, D. J., HARTMAN, C. A., HOEKSTRA, P. J., OOSTERLAAN, J., POLIER, G. V., KONRAD, K., VILARROYA, O., RAMOS-QUIROGA, J. A., SOLIVA, J. C., DURSTON, S., BUITELAAR, J. K., FARAONE, S. V., SHAW, P., THOMPSON, P. M. & FRANKE, B. 2017. Subcortical brain volume differences in participants with attention deficit hyperactivity disorder in children and adults: a cross-sectional mega-analysis. Lancet Psychiatry, 4, 310-319.

HUNG, P. L., LUI, C. C., LEE, C. C., CHIEN, Y. H., CHEN, F. S., CHEN, C. C., YU, H. R., CHUNG, M. Y. & HUANG, L. T. 2018. Gestational age, not transient hyperthyrotropinemia impacts brain white matter diffusion tensor imaging in premature infants. Experimental and Therapeutic Medicine, 15, 1013-1020.

HYDE, C., FUELSCHER, I., SCIBERRAS, E., EFRON, D., ANDERSON, V. A. & SILK, T. 2021. Understanding motor difficulties in children with ADHD: A fixel-based analysis of the corticospinal tract. Prog Neuropsychopharmacol Biol Psychiatry, 105, 110125.

JADDOE, V. W., MACKENBACH, J. P., MOLL, H. A., STEEGERS, E. A., TIEMEIER, H., VERHULST, F. C., WITTEMAN, J. C. & HOFMAN, A. 2006. The Generation R Study: Design and cohort profile. Eur J Epidemiol, 21, 475-84.

JAGANNATHAN, N. R., TANDON, N., RAGHUNATHAN, P. & KOCHUPILLAI, N. 1998. Reversal of abnormalities of myelination by thyroxine therapy in congenital hypothyroidism: localized in vivo proton magnetic resonance spectroscopy (MRS) study. Brain Research. Developmental Brain Research, 109, 179-86.

JANSEN, T. A., KOREVAAR, T. I. M., MULDER, T. A., WHITE, T., MUETZEL, R. L., PEETERS, R. P. & TIEMEIER, H. 2019. Maternal thyroid function during pregnancy and child brain morphology: a time window-specific analysis of a prospective cohort. Lancet Diabetes Endocrinol, 7, 629-637.

JULVEZ, J., ALVAREZ-PEDREROL, M., REBAGLIATO, M., MURCIA, M., FORNS, J., GARCIA-ESTEBAN, R., LERTXUNDI, N., ESPADA, M., TARDÓN, A., RIAÑO GALÁN, I. & SUNYER, J. 2013. Thyroxine levels during pregnancy in healthy women and early child neurodevelopment. Epidemiology, 24, 150-7.

KAPOOR, R., FANIBUNDA, S. E., DESOUZA, L. A., GUHA, S. K. & VAIDYA, V. A. 2015. Perspectives on thyroid hormone action in adult neurogenesis. J Neurochem, 133, 599-616.

KENNEDY, E., NIVINS, S., THOMPSON, B., MCKINLAY, C. J. D., HARDING, J. & TEAM, C. S. 2023. Neurodevelopmental correlates of caudate volume in children born at risk of neonatal hypoglycaemia. Pediatr Res, 93, 1634-1641.

KETY, S. & SCHMIDT, C. 1948. THE NITROUS OXIDE METHOD FOR THE QUANTITATIVE DETERMINATION OF CEREBRAL BLOOD FLOW IN MAN: THEORY, PROCEDURE AND NORMAL VALUES. J Clin Invest .

KLEIN, R. Z., SARGENT, J. D., LARSEN, P. R., WAISBREN, S. E., HADDOW, J. E. & MITCHELL, M. L. 2001. Relation of severity of maternal hypothyroidism to cognitive development of offspring. J Med Screen, 8, 18-20.

KOENIG, R. J. 1998. Thyroid hormone receptor coactivators and corepressors. Thyroid, 8, 703-13.

KOOISTRA, L., CRAWFORD, S., VAN BAAR, A. L., BROUWERS, E. P. & POP, V. J. 2006. Neonatal effects of maternal hypothyroxinemia during early pregnancy. Pediatrics, 117, 161-7.

KOOISTRA, L., STEMERDINK, N., VAN DER MEERE, J., VULSMA, T. & KALVERBOER, A. F. 2001. Behavioural correlates of early-treated congenital hypothyroidism. Acta Paediatr, 90, 1141-6.

KOOISTRA, L., VULSMA, T. & VAN DER MEERE, J. 2004. An investigation of impulsivity in children with early-treated congenital hypothyroidism. Dev Neuropsychol, 26, 595-610.

KOREVAAR, T. I. M., DERAKHSHAN, A., TAYLOR, P. N., MEIMA, M., CHEN, L., BLIDDAL, S., CARTY, D. M., MEEMS, M., VAIDYA, B., SHIELDS, B., GHAFOOR, F., POPOVA, P. V., MOSSO, L., OKEN, E., SUVANTO, E., HISADA, A., YOSHINAGA, J., BROWN, S. J., BASSOLS, J., AUVINEN, J., BRAMER, W. M., LÓPEZ-BERMEJO, A., DAYAN, C., BOUCAI, L., VAFEIADI, M., GRINEVA, E. N., TKACHUCK, A. S., POP, V. J. M., VRIJKOTTE, T. G., GUXENS, M., CHATZI, L., SUNYER, J., JIMÉNEZ-ZABALA, A., RIAÑO, I., MURCIA, M., LU, X., MUKHTAR, S., DELLES, C., FELDT-RASMUSSEN, U., NELSON, S. M., ALEXANDER, E. K., CHAKER, L., MÄNNISTÖ, T., WALSH, J. P., PEARCE, E. N., STEEGERS, E. A. P., PEETERS, R. P. & BIRTH, C. O. T. A. P. S. G. O. P. 2019. Association of Thyroid Function Test Abnormalities and Thyroid Autoimmunity With Preterm Birth: A Systematic Review and Meta-analysis. JAMA, 322, 632-641.

KOREVAAR, T. I. M., MEDICI, M., VISSER, T. J. & PEETERS, R. P. 2017. Thyroid disease in pregnancy: new insights in diagnosis and clinical management. Nat Rev Endocrinol, 13, 610-622.

KOREVAAR, T. I. M., MUETZEL, R., MEDICI, M., CHAKER, L., JADDOE, V. W. V., DE RIJKE, Y. B., STEEGERS, E. A. P., VISSER, T. J., WHITE, T., TIEMEIER, H. & PEETERS, R. P. 2016. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. Lancet Diabetes & Endocrinology, 4, 35-43.

KOUTSOKLENIS, A. & HONKASILTA, J. 2022. ADHD in the DSM-5-TR: What has changed and what has not. Front Psychiatry, 13, 1064141.

KRUITHOF, C. J., KOOIJMAN, M. N., VAN DUIJN, C. M., FRANCO, O. H., DE JONGSTE, J. C., KLAVER, C. C. W., MACKENBACH, J. P., MOLL, H. A., RAAT, H., RINGS, E., RIVADENEIRA, F., STEEGERS, E. A. P., TIEMEIER, H., UITTERLINDEN, A. G., VERHULST, F. C., WOLVIUS, E. B., HOFMAN, A. & JADDOE, V. W. V. 2014. The Generation R Study: Biobank update 2015. European Journal of Epidemiology, 29, 911-927.

LAFRANCHI, S. H., SNYDER, D. B., SESSER, D. E., SKEELS, M. R., SINGH, N., BRENT, G. A. & NELSON, J. C. 2003. Follow-up of newborns with elevated screening T4 concentrations. J Pediatr, 143, 296-301.

LAVADO-AUTRIC, R., AUSÓ, E., GARCÍA-VELASCO, J. V., ARUFE, M. E. C., ESCOBAR DEL REY, F., BERBEL, P. & MORREALE DE ESCOBAR, G. 2003. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest, 111, 1073-82.

LAZARUS, J. H., BESTWICK, J. P., CHANNON, S., PARADICE, R., MAINA, A., REES, R., CHIUSANO, E., JOHN, R., GUARALDO, V., GEORGE, L. M., PERONA, M., DALL'AMICO, D., PARKES, A. B., JOOMUN, M. & WALD, N. J. 2012. Antenatal thyroid screening and childhood cognitive function. N Engl J Med, 366, 493-501.

LEMKINE, G. F., RAJ, A., ALFAMA, G., TURQUE, N., HASSANI, Z., ALEGRIA-PRÉVOT, O., SAMARUT, J., LEVI, G. & DEMENEIX, B. A. 2005. Adult neural stem cell cycling in vivo requires thyroid hormone and its alpha receptor. FASEB J, 19, 863-5.

LEVIE, D., KOREVAAR, T. I. M., BATH, S. C., DALMAU-BUENO, A., MURCIA, M., ESPADA, M., DINEVA, M., IBARLUZEA, J. M., SUNYER, J., TIEMEIER, H., REBAGLIATO, M., RAYMAN, M. P., PEETERS, R. P. & GUXENS, M. 2018. Thyroid Function in Early Pregnancy, Child IQ, and Autistic Traits: A Meta-Analysis of Individual Participant Data. J Clin Endocrinol Metab, 103, 2967-2979.

LEVITON, A., PANETH, N., REUSS, M. L., SUSSER, M., ALLRED, E. N., DAMMANN, O., KUBAN, K., VAN MARTER, L. J. & PAGANO, M. 1999. Hypothyroxinemia of prematurity and the risk of cerebral white matter damage. J Pediatr, 134, 706-11.

LI, Y., SHAN, Z., TENG, W., YU, X., FAN, C., TENG, X., GUO, R., WANG, H., LI, J., CHEN, Y., WANG, W., CHAWINGA, M., ZHANG, L., YANG, L., ZHAO, Y. & HUA, T. 2010. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25-30 months. Clin Endocrinol (Oxf), 72, 825-9.

LISCHINSKY, J. E., SKOCIC, J., CLAIRMAN, H. & ROVET, J. 2016. Preliminary Findings show Maternal hypothyroidism May contribute to abnormal cortical Morphology in Offspring. Frontiers in Endocrinology, 7.

LIU, H., SHAN, Z., LI, C., MAO, J., XIE, X., WANG, W., FAN, C., WANG, H., ZHANG, H., HAN, C., WANG, X., LIU, X., FAN, Y., BAO, S. & TENG, W. 2014. Maternal subclinical hypothyroidism, thyroid autoimmunity, and the risk of miscarriage: a prospective cohort study. Thyroid, 24, 1642-9.

LIU, Y. C., YEH, C. T. & LIN, K. H. 2019. Molecular Functions of Thyroid Hormone Signaling in Regulation of Cancer Progression and Anti-Apoptosis. Int J Mol Sci, 20.

LORD, C. & RUTTER, M. 2003. Social communication questionnaire (SCQ). Torrance, CA: WPS.

LUCIA, F. S., PACHECO-TORRES, J., GONZALEZ-GRANERO, S., CANALS, S., OBREGON, M. J., GARCIA-VERDUGO, J. M. & BERBEL, P. 2018. Transient Hypothyroidism During Lactation Arrests Myelination in the Anterior Commissure of Rats. A Magnetic Resonance Image and Electron Microscope Study. Frontiers in Neuroanatomy, 12.

MA, T., LIAN, Z. C., QI, S. P., HEINZ, E. R. & DELONG, G. R. 1993. Magnetic resonance imaging of brain and the neuromotor disorder in endemic cretinism. Annals of Neurology, 34, 91-4.

MAIA, A. L., KIM, B. W., HUANG, S. A., HARNEY, J. W. & LARSEN, P. R. 2005. Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. J Clin Invest, 115, 2524-33.

MAKRIS, N., BUKA, S. L., BIEDERMAN, J., PAPADIMITRIOU, G. M., HODGE, S. M., VALERA, E. M., BROWN, A. B., BUSH, G., MONUTEAUX, M. C., CAVINESS, V. S., KENNEDY, D. N. & SEIDMAN, L. J. 2008. Attention and executive systems

abnormalities in adults with childhood ADHD: A DT-MRI study of connections. Cereb Cortex, 18, 1210-20.

MARTINEZ-GALAN, J. R., ESCOBAR DEL REY, F., MORREALE DE ESCOBAR, G., SANTACANA, M. & RUIZ-MARCOS, A. 2004. Hypothyroidism alters the development of radial glial cells in the term fetal and postnatal neocortex of the rat. Brain Res Dev Brain Res, 153, 109-14.

MARTINEZ-GALAN, J. R., PEDRAZA, P., SANTACANA, M., ESCOBAR DEL REY, F., MORREALE DE ESCOBAR, G. & RUIZ-MARCOS, A. 1997. Myelin basic protein immunoreactivity in the internal capsule of neonates from rats on a low iodine intake or on methylmercaptoimidazole (MMI). Brain Res Dev Brain Res, 101, 249-56.

MARTÍNEZ-GALÁN, J. R., PEDRAZA, P., SANTACANA, M., ESCOBAR DEL RAY, F., MORREALE DE ESCOBAR, G. & RUIZ-MARCOS, A. 1997. Early effects of iodine deficiency on radial glial cells of the hippocampus of the rat fetus. A model of neurological cretinism. J Clin Invest, 99, 2701-9.

MAYERL, S., VISSER, T. J., DARRAS, V. M., HORN, S. & HEUER, H. 2012. Impact of Oatp1c1 deficiency on thyroid hormone metabolism and action in the mouse brain. Endocrinology, 153, 1528-37.

MILLAR, L. K., WING, D. A., LEUNG, A. S., KOONINGS, P. P., MONTORO, M. N. & MESTMAN, J. H. 1994. Low birth weight and preeclampsia in pregnancies complicated by hyperthyroidism. Obstet Gynecol, 84, 946-9.

MILLER, B. L. 1991. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed, 4, 47-52.

MODESTO, T., TIEMEIER, H., PEETERS, R. P., JADDOE, V. W., HOFMAN, A., VERHULST, F. C. & GHASSABIAN, A. 2015. Maternal Mild Thyroid Hormone Insufficiency in Early Pregnancy and Attention-Deficit/Hyperactivity Disorder Symptoms in Children. JAMA Pediatr, 169, 838-45.

MORAN, C. & CHATTERJEE, K. 2015. Resistance to thyroid hormone due to defective thyroid receptor alpha. Best Pract Res Clin Endocrinol Metab, 29, 647-57.

MORAN, C., SCHOENMAKERS, N., VISSER, W. E., SCHOENMAKERS, E., AGOSTINI, M. & CHATTERJEE, K. 2022. Genetic disorders of thyroid development, hormone biosynthesis and signalling. Clin Endocrinol (Oxf), 97, 502-514.

MORREALE DE ESCOBAR, G., OBREGON, M. J. & ESCOBAR DEL REY, F. 2004. Role of thyroid hormone during early brain development. Eur J Endocrinol, 151 Suppl 3, U25-37.

MORREALE DE ESCOBAR, G., OBREGÓN, M. J. & ESCOBAR DEL REY, F. 2000. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J Clin Endocrinol Metab, 85, 3975-87.

MORTE, B., MANZANO, J., SCANLAN, T., VENNSTRÖM, B. & BERNAL, J. 2002. Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Proc Natl Acad Sci U S A, 99, 3985-9.

MORTE B, BERNAL J. Thyroid hormone action: astrocyte-neuron communication. Front Endocrinol (Lausanne). 2014;5:82. Published 2014 May 30. doi:10.3389/fendo.2014.00082

MULLER, I., TAYLOR, P. N., DANIEL, R. M., HALES, C., SCHOLZ, A., CANDLER, T., PETTIT, R. J., EVANS, W. D., SHILLABEER, D., DRAMAN, M. S., DAYAN, C. M., TANG, H. K. C., OKOSIEME, O., GREGORY, J. W., LAZARUS, J. H., REES, D. A. & LUDGATE, M. E. 2020. CATS II Long-term Anthropometric and Metabolic Effects of Maternal Sub-optimal Thyroid Function in Offspring and Mothers. J Clin Endocrinol Metab, 105.

NAVARRO, D., ALVARADO, M., NAVARRETE, F., GINER, M., OBREGON, M. J., MANZANARES, J. & BERBERL, P. 2015. Gestational and early postnatal hypothyroidism alters VGIuT1 and VGAT bouton distribution in the neocortex and hippocampus, and behavior in rats. Frontiers in Neuroanatomy, 9.

NG, L., LIU, H., ST GERMAIN, D. L., HERNANDEZ, A. & FORREST, D. 2017. Deletion of the Thyroid Hormone-Activating Type 2 Deiodinase Rescues Cone Photoreceptor Degeneration but Not Deafness in Mice Lacking Type 3 Deiodinase. Endocrinology, 158, 1999-2010.

NG, S. M., TURNER, M. A., GAMBLE, C., DIDI, M., VICTOR, S., ATKINSON, J., SLUMING, V., PARKES, L. M., TIETZE, A., ABERNETHY, L. J. & WEINDLING, A. M. 2014. Effect of thyroxine on brain microstructure in extremely premature babies: magnetic resonance imaging findings in the TIPIT study. Pediatric Radiology, 44, 987-996.

NG, S. M., TURNER, M. A., GAMBLE, C., DIDI, M., VICTOR, S., MANNING, D., SETTLE, P., GUPTA, R., NEWLAND, P. & WEINDLING, A. M. 2013. An explanatory randomised placebo controlled trial of levothyroxine supplementation for babies born <28 weeks' gestation: results of the TIPIT trial. Trials, 14, 211.

NG, S. M., TURNER, M. A. & WEINDLING, A. M. 2020. Neurodevelopmental Outcomes at 42 Months After Thyroxine Supplementation in Infants Below 28 Weeks' Gestation: A Randomized Controlled Trial. Thyroid, 30, 948-954.

OGAWA, S., LEE, T. M., KAY, A. R. & TANK, D. W. 1990. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci U S A, 87, 9868-72.

OKOSIEME, O. E., KHAN, I. & TAYLOR, P. N. 2018. Preconception management of thyroid dysfunction. Clin Endocrinol (Oxf), 89, 269-279.

OKOSIEME, O. E., USMAN, D., TAYLOR, P. N., DAYAN, C. M., LYONS, G., MORAN, C., CHATTERJEE, K. & REES, D. A. 2023. Cardiovascular morbidity and mortality in patients in Wales, UK with resistance to thyroid hormone β (RTHβ): a linked-record cohort study. Lancet Diabetes Endocrinol, 11, 657-666.

OOKUBO, M., SADAMATSU, M., YOSHIMURA, A., SUZUKI, S., KATO, N., KOJIMA, H., YAMADA, N. & KANAI, H. 2015. Aberrant Monoaminergic System in Thyroid Hormone Receptor-β Deficient Mice as a Model of Attention-Deficit/Hyperactivity Disorder. Int J Neuropsychopharmacol, 18, pyv004.

OTTERMAN, D. L., KOOPMAN-VERHOEFF, M. E., WHITE, T. J., TIEMEIER, H., BOLHUIS, K. & JANSEN, P. W. 2019. Executive functioning and neurodevelopmental disorders in early childhood: a prospective population-based study. Child Adolesc Psychiatry Ment Health, 13, 38.

PANAGIOTOU, G., TAYLOR, P. N., REES, D. A. & OKOSIEME, O. E. 2022. Late offspring effects of antenatal thyroid screening. Br Med Bull, 143, 16-29.

PHAROAH, P. O. & CONNOLLY, K. J. 1991. Effects of maternal iodine supplementation during pregnancy. Arch Dis Child, 66, 145-7.

PHAROAH, P. O., ELLIS, S. M., EKINS, R. P. & WILLIAMS, E. S. 1976. Maternal thyroid function, iodine deficiency and fetal development. Clin Endocrinol (Oxf), 5, 159-66.

PINE-TWADDELL, E., ROMERO, C. J. & RADOVICK, S. 2013. Vertical transmission of hypopituitarism: critical importance of appropriate interpretation of thyroid function tests and levothyroxine therapy during pregnancy. Thyroid, 23, 892-7.

POP, V. J., BROUWERS, E. P., VADER, H. L., VULSMA, T., VAN BAAR, A. L. & DE VIJLDER, J. J. 2003. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. Clin Endocrinol (Oxf), 59, 282-8.

PÄKKILÄ, F., MÄNNISTÖ, T., HARTIKAINEN, A. L. & SUVANTO, E. 2018. Maternal Thyroid Function During Pregnancy and the Child's Linguistic and Sensory Development in the Northern Finland Birth Cohort 1986. Front Endocrinol (Lausanne), 9, 127.

PÄKKILÄ, F., MÄNNISTÖ, T., POUTA, A., HARTIKAINEN, A. L., RUOKONEN, A., SURCEL, H. M., BLOIGU, A., VÄÄRÄSMÄKI, M., JÄRVELIN, M. R., MOILANEN, I. & SUVANTO, E. 2014. The impact of gestational thyroid hormone concentrations on ADHD symptoms of the child. J Clin Endocrinol Metab, 99, E1-8.

QUERIDO, A. 1975. Endemic cretinism--a continuous personal educational experience during 10 years. Postgrad Med J, 51, 591-9.

RACHMIEL, M., BLASER, S., WIDJAJA, E. & ROVET, J. 2013. Children with Congenital Hypothyroidism Have Similar Neuroradiological Abnormal Findings as Healthy Ones. Scientific World Journal.

RADETTI, G., GENTILI, L., PAGANINI, C., OBERHOFER, R., DELUGGI, I. & DELUCCA, A. 2000. Psychomotor and audiological assessment of infants born to

mothers with subclinical thyroid dysfunction in early pregnancy. Minerva Pediatr, 52, 691-8.

RATNANATHER, J. T. 2020. Structural neuroimaging of the altered brain stemming from pediatric and adolescent hearing loss-Scientific and clinical challenges. Wiley Interdiscip Rev Syst Biol Med, 12, e1469.

REMERAND, G., BOESPFLUG-TANGUY, O., TONDUTI, D., TOURAINE, R., RODRIGUEZ, D., CURIE, A., PERRETON, N., DES PORTES, V., SARRET, C. & GROUP, R. A. S. 2019. Expanding the phenotypic spectrum of Allan-Herndon-Dudley syndrome in patients with SLC16A2 mutations. Dev Med Child Neurol, 61, 1439-1447.

ROGGE, B., HELDMANN, M., CHATTERJEE, K., MORAN, C., GÖTTLICH, M., UTER, J., WAGNER-ALTENDORF, T. A., STEINHARDT, J., BRABANT, G., MÜNTE, T. F. & CIRKEL, A. 2023. Changes in brain structure in subjects with resistance to thyroid hormone due to THRB mutations. Thyroid Res, 16, 34.

ROMÁN, G. C., GHASSABIAN, A., BONGERS-SCHOKKING, J. J., JADDOE, V. W., HOFMAN, A., DE RIJKE, Y. B., VERHULST, F. C. & TIEMEIER, H. 2013. Association of gestational maternal hypothyroxinemia and increased autism risk. Ann Neurol, 74, 733-42.

ROSEN, R. D. & SAPRA, A. 2019. Embryology, Thyroid.

ROVET, J. F. 1999. Congenital hypothyroidism: long-term outcome. Thyroid, 9, 741-8.

ROVET, J. F. & HEPWORTH, S. 2001. Attention problems in adolescents with congenital hypothyroidism: a multicomponential analysis. J Int Neuropsychol Soc, 7, 734-44.

ROY, C. & SHERRINGTON, C. 1890. On the Regulation of the Blood-supply of the Brain. J Physiology.

SAMADI, A., SKOCIC, J. & ROVET, J. F. 2015. Children born to women treated for hypothyroidism during pregnancy show abnormal corpus callosum development. Thyroid, 25, 494-502.

SCRATCH, S. E., HUNT, R. W., THOMPSON, D. K., AHMADZAI, Z. M., DOYLE, L. W., INDER, T. E. & ANDERSON, P. J. 2014. Free thyroxine levels after very preterm birth and neurodevelopmental outcomes at age 7 years. Pediatrics, 133, e955-63.

SEGNI, M. 2017. Disorders of the Thyroid Gland in Infancy, Childhood and Adolescence. In: FEINGOLD, K. R., ANAWALT, B., BOYCE, A., CHROUSOS, G., DE HERDER, W. W., DHATARIYA, K., DUNGAN, K., HERSHMAN, J. M., HOFLAND, J., KALRA, S., KALTSAS, G., KOCH, C., KOPP, P., KORBONITS, M., KOVACS, C. S., KUOHUNG, W., LAFERRÈRE, B., LEVY, M., MCGEE, E. A., MCLACHLAN, R., MORLEY, J. E., NEW, M., PURNELL, J., SAHAY, R., SINGER, F., SPERLING, M. A., STRATAKIS, C. A., TRENCE, D. L. & WILSON, D. P. (eds.) Endotext. South Dartmouth (MA): MDText.com, Inc.

SHAAG, A., ANIKSTER, Y., CHRISTENSEN, E., GLUSTEIN, J. Z., FOIS, A., MICHELAKAKIS, H., NIGRO, F., PRONICKA, E., RIBES, A. & ZABOT, M. T. 1995. The molecular basis of canavan (aspartoacylase deficiency) disease in European non-Jewish patients. Am J Hum Genet, 57, 572-80.

SHARLIN, D. S., TIGHE, D., GILBERT, M. E. & ZOELLER, R. T. 2008. The balance between oligodendrocyte and astrocyte production in major white matter tracts is linearly related to serum total thyroxine. Endocrinology, 149, 2527-36.

SHAW, P., MALEK, M., WATSON, B., GREENSTEIN, D., DE ROSSI, P. & SHARP, W. 2013. Trajectories of cerebral cortical development in childhood and adolescence and adult attention-deficit/hyperactivity disorder. Biol Psychiatry, 74, 599-606.

SHIELDS, B. M., KNIGHT, B. A., HILL, A., HATTERSLEY, A. T. & VAIDYA, B. 2011. Fetal thyroid hormone level at birth is associated with fetal growth. J Clin Endocrinol Metab, 96, E934-8.

SIESSER, W. B., ZHAO, J., MILLER, L. R., CHENG, S. Y. & MCDONALD, M. P. 2006. Transgenic mice expressing a human mutant beta1 thyroid receptor are hyperactive, impulsive, and inattentive. Genes Brain Behav, 5, 282-97.

SOARES, J. M., MARQUES, P., ALVES, V. & SOUSA, N. 2013. A hitchhiker's guide to diffusion tensor imaging. Front Neurosci, 7, 31.

STAGNARO-GREEN, A., DONG, A. & STEPHENSON, M. D. 2020. Universal screening for thyroid disease during pregnancy should be performed. Best Pract Res Clin Endocrinol Metab, 34, 101320.

STEIN, J. L., MEDLAND, S. E., VASQUEZ, A. A., HIBAR, D. P., SENSTAD, R. E., WINKLER, A. M., TORO, R., APPEL, K., BARTECEK, R., BERGMANN, Ø., BERNARD, M., BROWN, A. A., CANNON, D. M., CHAKRAVARTY, M. M., CHRISTOFOROU, A., DOMIN, M., GRIMM, O., HOLLINSHEAD, M., HOLMES, A. J., HOMUTH, G., HOTTENGA, J. J., LANGAN, C., LOPEZ, L. M., HANSELL, N. K., HWANG, K. S., KIM, S., LAJE, G., LEE, P. H., LIU, X., LOTH, E., LOURDUSAMY, A., MATTINGSDAL, M., MOHNKE, S., MANIEGA, S. M., NHO, K., NUGENT, A. C., O'BRIEN, C., PAPMEYER, M., PÜTZ, B., RAMASAMY, A., RASMUSSEN, J., RIJPKEMA, M., RISACHER, S. L., RODDEY, J. C., ROSE, E. J., RYTEN, M., SHEN, L., SPROOTEN, E., STRENGMAN, E., TEUMER, A., TRABZUNI, D., TURNER, J., VAN EIJK, K., VAN ERP, T. G., VAN TOL, M. J., WITTFELD, K., WOLF, C., WOUDSTRA, S., ALEMAN, A., ALHUSAINI, S., ALMASY, L., BINDER, E. B., BROHAWN, D. G., CANTOR, R. M., CARLESS, M. A., CORVIN, A., CZISCH, M., CURRAN, J. E., DAVIES, G., DE ALMEIDA, M. A., DELANTY, N., DEPONDT, C., DUGGIRALA, R., DYER, T. D., ERK, S., FAGERNESS, J., FOX, P. T., FREIMER, N. B., GILL, M., GÖRING, H. H., HAGLER, D. J., HOEHN, D., HOLSBOER, F., HOOGMAN, M., HOSTEN, N., JAHANSHAD, N., JOHNSON, M. P., KASPERAVICIUTE, D., KENT, J. W., JR., KOCHUNOV, P., LANCASTER, J. L., LAWRIE, S. M., LIEWALD, D. C., MANDL, R., MATARIN, M., MATTHEISEN, M., MEISENZAHL, E., MELLE, I., MOSES, E. K., MÜHLEISEN, T. W., et al. 2012. Identification of common variants associated with human hippocampal and intracranial volumes. Nat Genet, 44, 552-61.

STENZEL, D. & HUTTNER, W. B. 2013. Role of maternal thyroid hormones in the developing neocortex and during human evolution. Front Neuroanat, 7, 19.

STEPHENS, R. J., CHUNG, S. A., JOVANOVIC, D., GUERRA, R., STEPHENS, B., SANDOR, P. & SHAPIRO, C. M. 2013. Relationship between polysomnographic sleep architecture and behavior in medication-free children with TS, ADHD, TS and ADHD, and controls. Journal of Developmental & Behavioral Pediatrics, 34, 688-696.

STILES, J. & JERNIGAN, T. L. 2010. The basics of brain development. Neuropsychol Rev, 20, 327-48.

STROMME, P., GROENEWEG, S., DE SOUZA, E. C. L., ZEVENBERGEN, C., TORGERSBRATEN, A., HOLMGREN, A., GURCAN, E., MEIMA, M. E., PEETERS, R. P., VISSER, W. E., JOHANSSON, L. H., BABOVIC, A., ZETTERBERG, H., HEUER, H., FRENGEN, E., MISCEO, D. & VISSER, T. J. 2018. Mutated Thyroid Hormone Transporter OATP1C1 Associates with Severe Brain Hypometabolism and Juvenile Neurodegeneration. Thyroid, 28, 1406-1415.

SU, P. Y., HUANG, K., HAO, J. H., XU, Y. Q., YAN, S. Q., LI, T., XU, Y. H. & TAO, F. B. 2011. Maternal thyroid function in the first twenty weeks of pregnancy and subsequent fetal and infant development: a prospective population-based cohort study in China. J Clin Endocrinol Metab, 96, 3234-41.

TANAKA-ARAKAWA, M. M., MATSUI, M., TANAKA, C., UEMATSU, A., UDA, S., MIURA, K., SAKAI, T. & NOGUCHI, K. 2015. Developmental changes in the corpus callosum from infancy to early adulthood: a structural magnetic resonance imaging study. PLoS One, 10, e0118760.

TAYLOR, S. J., WHINCUP, P. H., HINDMARSH, P. C., LAMPE, F., ODOKI, K. & COOK, D. G. 2001. Performance of a new pubertal self-assessment questionnaire: a preliminary study. Paediatr Perinat Epidemiol, 15, 88-94.

TAYLOR, P. N., ALBRECHT, D., SCHOLZ, A., GUTIERREZ-BUEY, G., LAZARUS, J. H., DAYAN, C. M. & OKOSIEME, O. E. 2018. Global epidemiology of hyperthyroidism and hypothyroidism. Nat Rev Endocrinol, 14, 301-316.

TEAM, R. C. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. [Online]. https://www.R-project.org. [Accessed].

TEN DONKELAAR, H. 2015. Development of the basal ganglia and the basal forebrain.

THAPAR, A. & COOPER, M. 2016. Attention deficit hyperactivity disorder. Lancet, 387, 1240-50.

THAPAR, A., COOPER, M. & RUTTER, M. 2017. Neurodevelopmental disorders. Lancet Psychiatry, 4, 339-346.

THAPAR, A., HARRINGTON, R., ROSS, K. & MCGUFFIN, P. 2000. Does the definition of ADHD affect heritability? J Am Acad Child Adolesc Psychiatry, 39, 1528-36.

THOMPSON, W., RUSSELL, G., BARAGWANATH, G., MATTHEWS, J., VAIDYA, B. & THOMPSON-COON, J. 2018. Maternal thyroid hormone insufficiency during pregnancy and risk of neurodevelopmental disorders in offspring: A systematic review and meta-analysis. Clin Endocrinol (Oxf), 88, 575-584.

TOLOZA, F. J. K., DERAKHSHAN, A., MÄNNISTÖ, T., BLIDDAL, S., POPOVA, P. V., CARTY, D. M., CHEN, L., TAYLOR, P., MOSSO, L., OKEN, E., SUVANTO, E., ITOH, S., KISHI, R., BASSOLS, J., AUVINEN, J., LÓPEZ-BERMEJO, A., BROWN, S. J., BOUCAI, L., HISADA, A., YOSHINAGA, J., SHILOVA, E., GRINEVA, E. N., VRIJKOTTE, T. G. M., SUNYER, J., JIMÉNEZ-ZABALA, A., RIAÑO-GALAN, I., LOPEZ-ESPINOSA, M. J., PROKOP, L. J., SINGH OSPINA, N., BRITO, J. P., RODRIGUEZ-GUTIERREZ, R., ALEXANDER, E. K., CHAKER, L., PEARCE, E. N., PEETERS, R. P., FELDT-RASMUSSEN, U., GUXENS, M., CHATZI, L., DELLES, C., ROETERS VAN LENNEP, J. E., POP, V. J. M., LU, X., WALSH, J. P., NELSON, S. M., KOREVAAR, T. I. M. & MARAKA, S. 2022. Association between maternal thyroid function and risk of gestational hypertension and pre-eclampsia: a systematic review and individual-participant data meta-analysis. Lancet Diabetes Endocrinol, 10, 243-252.

UTER, J., HELDMANN, M., ROGGE, B., OBST, M., STEINHARDT, J., BRABANT, G., MORAN, C., CHATTERJEE, K. & MÜNTE, T. F. 2020. Patients with mutations of the Thyroid hormone beta-receptor show an ADHD-like phenotype for performance monitoring: an electrophysiological study. Neuroimage Clin, 26, 102250.

VAIDYA, B., ANTHONY, S., BILOUS, M., SHIELDS, B., DRURY, J., HUTCHISON, S. & BILOUS, R. 2007. Detection of thyroid dysfunction in early pregnancy: Universal screening or targeted high-risk case finding? J Clin Endocrinol Metab, 92, 203-7.

VALERA, E. M., FARAONE, S. V., MURRAY, K. E. & SEIDMAN, L. J. 2007. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. Biol Psychiatry, 61, 1361-9.

VAN DER SPEK, A. H., FLIERS, E. & BOELEN, A. 2017. The classic pathways of thyroid hormone metabolism. Mol Cell Endocrinol, 458, 29-38.

VAN DER WEIJDEN, C. W. J., BIONDETTI, E., GUTMANN, I. W., DIJKSTRA, H., MCKERCHAR, R., DE PAULA FARIA, D., DE VRIES, E. F. J., MEILOF, J. F., DIERCKX, R. A. J. O., PREVOST, V. H. & RAUSCHER, A. 2023. Quantitative myelin imaging with MRI and PET: an overview of techniques and their validation status. Brain, 146, 1243-1266.

VAN GEEST, F. S., GROENEWEG, S. & VISSER, W. E. 2021. Monocarboxylate transporter 8 deficiency: update on clinical characteristics and treatment. Endocrine, 71, 689-695.

VELA, A., PÉREZ-NANCLARES, G., RÍOS, I., RICA, I., PORTILLO, N., CASTAÑO, L. & RTH, S. G. F. T. S. O. 2019. Thyroid hormone resistance from newborns to adults: a Spanish experience. J Endocrinol Invest, 42, 941-949.

VENTURI, S., DONATI, F. M., VENTURI, A. & VENTURI, M. 2000. Environmental iodine deficiency: A challenge to the evolution of terrestrial life? Thyroid, 10, 727-9.

VERMIGLIO, F., LO PRESTI, V. P., MOLETI, M., SIDOTI, M., TORTORELLA, G., SCAFFIDI, G., CASTAGNA, M. G., MATTINA, F., VIOLI, M. A., CRISÀ, A., ARTEMISIA, A. & TRIMARCHI, F. 2004. Attention deficit and hyperactivity disorders in the offspring of mothers exposed to mild-moderate iodine deficiency: a possible novel iodine deficiency disorder in developed countries. J Clin Endocrinol Metab, 89, 6054-60.

VION-DURY, J., MEYERHOFF, D. J., COZZONE, P. J. & WEINER, M. W. 1994. What might be the impact on neurology of the analysis of brain metabolism by in vivo magnetic resonance spectroscopy? J Neurol, 241, 354-71.

VOLKOW, N. D., WANG, G. J., NEWCORN, J. H., KOLLINS, S. H., WIGAL, T. L., TELANG, F., FOWLER, J. S., GOLDSTEIN, R. Z., KLEIN, N., LOGAN, J., WONG,

C. & SWANSON, J. M. 2011. Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway. Mol Psychiatry, 16, 1147-54.

WASSERMAN, E. E., NELSON, K., ROSE, N. R., EATON, W., PILLION, J. P., SEABERG, E., TALOR, M. V., BUREK, L., DUGGAN, A. & YOLKEN, R. H. 2008. Maternal thyroid autoantibodies during the third trimester and hearing deficits in children: an epidemiologic assessment. Am J Epidemiol, 167, 701-10.

WEJAPHIKUL, K., GROENEWEG, S., HILHORST-HOFSTEE, Y., CHATTERJEE, V. K., PEETERS, R. P., MEIMA, M. E. & VISSER, W. E. 2019. Insight Into Molecular Determinants of T3 vs T4 Recognition From Mutations in Thyroid Hormone Receptor α and β. J Clin Endocrinol Metab, 104, 3491-3500.

WHEELER, S. M., MCANDREWS, M. P., SHEARD, E. D. & ROVET, J. 2012. Visuospatial Associative Memory and Hippocampal Functioning in Congenital Hypothyroidism. Journal of the International Neuropsychological Society, 18, 49-56.

WHEELER, S. M., MCLELLAND, V. C., SHEARD, E., MCANDREWS, M. P. & ROVET, J. F. 2015. Hippocampal functioning and verbal associative memory in adolescents with congenital hypothyroidism. Frontiers in Endocrinology, 6.

WHEELER, S. M., WILLOUGHBY, K. A., MCANDREWS, M. P. & ROVET, J. F. 2011. Hippocampal Size and Memory Functioning in Children and Adolescents with Congenital Hypothyroidism. Journal of Clinical Endocrinology & Metabolism, 96, E1427-E1434.

WHITE, T., MUETZEL, R. L., EL MARROUN, H., BLANKEN, L. M. E., JANSEN, P., BOLHUIS, K., KOCEVSKA, D., MOUS, S. E., MULDER, R., JADDOE, V. W. V., VAN DER LUGT, A., VERHULST, F. C. & TIEMEIER, H. 2018. Paediatric population neuroimaging and the Generation R Study: the second wave. European Journal of Epidemiology, 33, 99-125.

WILLOUGHBY, K. A., MCANDREWS, M. P. & ROVET, J. F. 2014a. Accuracy of episodic autobiographical memory in children with early thyroid hormone deficiency using a staged event. Developmental Cognitive Neuroscience, 9, 1-11.

WILLOUGHBY, K. A., MCANDREWS, M. P. & ROVET, J. F. 2014b. Effects of Maternal Hypothyroidism on Offspring Hippocampus and Memory. Thyroid, 24, 576-584.

WINKLEWSKI, P. J., SABISZ, A., NAUMCZYK, P., JODZIO, K., SZUROWSKA, E. & SZARMACH, A. 2018. Understanding the Physiopathology Behind Axial and Radial Diffusivity Changes-What Do We Know? Front Neurol, 9, 92.

YAEL, D., TAHARY, O., GUROVICH, B., BELELOVSKY, K. & BAR-GAD, I. 2019. Disinhibition of the Nucleus Accumbens Leads to Macro-Scale Hyperactivity Consisting of Micro-Scale Behavioral Segments Encoded by Striatal Activity. J Neurosci, 39, 5897-5909.

ZENDEL, B. R., WILLOUGHBY, K. A. & ROVET, J. F. 2013. Neuroplastic effects of music lessons on hippocampal volume in children with congenital hypothyroidism. Neuroreport, 24, 947-50.

ZIMMERMANN, M. B. 2008. Research on iodine deficiency and goiter in the 19th and early 20th centuries. J Nutr, 138, 2060-3.

ZUÑIGA, L. F. F., MUÑOZ, Y. S. & PUSTOVRH, M. C. 2022. Thyroid hormones: Metabolism and transportation in the fetoplacental unit. Mol Reprod Dev, 89, 526-539.

Chapter 7

Chapter 7: Appendix

- 7.1 History of iodine and cretinism
- 7.2.PRISMA checklist
- 7.3 Search criteria for systematic review
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7.1 History of iodine and cretinism

Iodine

Iodine is an element and essential micronutrient, with adequate access for children and pregnant women included in the United Nations Convention on the Rights of the Child Article 24, part 2 (2013). Iodine containing thyroid hormones/ precursors are found unanimously throughout the animal kingdom, demonstrating the key role of iodine and thyroid function in bodily processes (Stenzel & Huttner, 2013).

The majority of the iodine in the body is metabolised within the thyroid gland and incorporated into thyroid hormones themselves (Dunn, 1998). Iodine is also found in several other tissues, such as mammary glands, gastric mucosa, and the eye, but its role is unclear (Ahad, 2010; Venturi, 2001). Iodine is predominantly found in the ocean and the soil of seaside areas. The soil of many inland and mountain areas are now leeched of iodine, and as such, much lower quantities are available in the local diet. If the soil is iodine rich, iodine can be taken up and absorbed from fruit and vegetables, grains and milk.

Since Roman times, travellers to the Swiss Alps have commented on the presence of goitres. Also noted in the Andes. Until the last few decades, the majority of large population cohort studies about iodine deficiency were based on goitres, as they are easy and quick to assess, so also more affordable and easier to conduct on a large scale compared to blood tests or measuring the very variable amount of iodine in urine. Iodine was identified as an element in the 1800s, but in Ancient Greece, links between marine sponges, which we now know are iodine rich, and treatment of goitres, have been recorded. Galen and marine sponges. This association was also continued in medieval Italy. De Villanova noted sponges were only successful in treating goitre in younger people, with goitre of recent origin, not large chronic goitres.

It was the recognition of the effect of iodine or lack of on goitres that led to salt iodinisation programmes. Iodinisation of salt in a large proportion of the world has been revolutionary in preventing iodine deficiency disorders, from simply unsightly goitres to the devastating effects of neurological cretinism (Ahad, 2010).

Appendix

Cretinism

In Bremis, a village of the Valais, as I have seen myself… it is usual that many infants suffer from [innate folly]. Besides, the head is sometimes misshapen ; the tongue is huge and swollen ; they are dumb; the throat is often goitrous. Thus they present an ugly sight; and sitting in the streets and looking into the sun, and putting little sticks in between their fingers, twisting their bodies in various ways, with their mouths agape they provoke passersby to laughter and astonishment.*

*Today, Bramois or Brämis.

Felix Platter, 1602, Swiss physician and psychiatrist.

Reference: Felix Platter [Platerus, Plater], Swiss physician and psychiatrist, Praxeos, seu de cognoscendis, praedicendis, praeca- vendis curandisque affectibus homini incommodantibus tractatus . . . , Basel : Conrad Waldkirch, 1602-1608, vol. 1, 95-96.

Table 1: history of the accumulation of evidence for the importance of iodine and thyroid function during pregnancy and the potentially detrimental effect on offspring

Table adapted and updated from literature and reviews by Morreale de Escobar (2004) and Zimmerman (2008), and https://www.jstor.org/stable/pdf/44446877.pdf?refreqid=excelsior%3A5f77c83fb5d6c6080b9c5c443b607c59&ab_segments=&origin=&initiator=&acceptTC=1

BULLETIN OF THE HISTORY OF MEDICINE ORGAN OF THE AMERICAN ASSOCIATION FOR THE HISTORY OF MEDICINE AND OF THE JOHNS HOPKINS INSTITUTE OF THE HISTORY OF MEDICINE VOLUME XXXVI NOVEMBER-DECEMBER, 1962 NUMBER 6 THE DISCOVERY OF CRETINISM 1 PAUL F. CRANEFIELD

Year	Author	Contribution
1811	Courtois	Iodine crystals created, soon identified as a new element.

Appendix

1821	Coindet	Suggestion it was the iodine in seaweed that made seaweed effective in the treatment of goitres.
1825-1835	Boussingault and Roulin	Reports from the Andes, that areas with few goitre cases had iodine in the rock salt. Demonstrated reversal of some goitres in people given rock salt with iodine in. Iodine proposed as a potential treatment, but its lack not recognised as the cause.
1850	Curling	Description of congenital hypothyroidism and effect on neurodevelopment- two children without thyroids.
1851	Chatin	First published hypothesis that iodine deficiency was the cause of goitre, including Boussingault's reports as evidence. Met with sceptiscism.
1896 1897	Baumann Roos	Iodine found in the thyroid gland, establishing the link between lack of iodine, goitre and myxoedema (hypothyroidism).
1915	Hunziker-Schild	Swiss Alpine experience of severe endemic goitre with cretinism. He made a good deduction that although it is not known whether the foetus makes its own TH or gets it from its mother, it seems likely it does receive it from its mother in the womb, as reflected with children born without thyroids that are born in a normal state but then develop myxoedematous signs in the months following. Also touched on prophylactic measures.

Appendix

1915-1919	Marine & Kimball	Iodine prophylaxis studies in the Midwest of the USA.
1918	Bayard	Iodised salt for goitre **** get more detail from Zimmerman
1917	McCarrison	Himalayas. Famous quote “it is of the greatest importance to inquire into the antenatal history of all backward children and to examine the mother for thyroid defect”. He described the myxoedematous type of cretinism
1920s		In part of Switzerland, in 1922, the first public health intervention programme created to eradicate newborn goitre and endemic cretinism. In 1924 Michigan was the first state in the USA to start iodinsing salt state wide.
1960s-1970s	Choufoer 1965, Pharoah 1971 & 1991	Papua New Guinea & Andean regions. Researchers noted pregnant women in ID areas were unable to respond at onset of pregnancy with increase in circulating thyroxine – unlike that physiological response to gestation seen in women with adequate iodine intake. Interventions EARLY in pregnancy with replacing iodine before end of 2nd trimester prevented birth of cretins. Also found the non-cretin inhabitants of the region had an increase in IQ post replacement.This recognition of the important social and economic effects for the whole local population created world-wide impetus to address iodine dietary deficiency.
1990	Mandel	Evidence for increasing thyroxine need during pregnancy in 12 known hypothyroid women. Their conclusion was that hypothyroid women should be monitored during pregnancy.
1992	Vitti	Evidence that even mild ID is potentially damaging for neurodevelopment in early pregnancy.

1994	Cao	Studied the effect of the timing of treatment for iodine deficiency on neurological outcomes of offspring in pregnant women. “Up to the end of the second trimester, iodine treatment protects the fetal brain from the effects of iodine deficiency. Treatment later in pregnancy or after delivery may improve brain growth and developmental achievement slightly, but it does not improve neurologic status.”
1999	Haddow	Analysed the stored antenatal serum samples of 25,216 women then recruited to undergo cognitive assessment. Sixty two women were found to have thyroid deficiency, 48 of which who were not treated for the condition during the pregnancy under study. 47 of the women had thyrotropin levels at or above the 99.7th centile. The full-scale IQ scores of their children averaged 7 points lower than those of the 124 matched control children (P=0.005); nineteen percent had scores of 85 or less.
2003	Pop	Found children of hypothyroxinaemic mothers at gestational age 12 weeks with further decrease in T4 later in pregnancy mothers’ was associated with neurodevelopmental delays at age 2 years old, with reduced mental and motor scores. Those hypothroxinaemic mothers whose fT4 levels increased in the second and third trimesters were similar to controls. However, it is difficult to judge these assessments at age of one year old and use it to predict future development, especially in a small study population (<60 in each group of cases and controls).
2010	Henrichs	Part of the large prospective population cohort Generation R study. >3000 mother-child pairs with data on the maternal thyroid function at 13 weeks gestation. A number of language and developmental assessments

		were undertaken at age 30 months. Expressive language delay was associated with mild and severe maternal hypothyroxinemia. Severe maternal hypothyroxinemia also predicted a higher risk of nonverbal cognitive delay (OR = 2.03; 95% CI = 1.22-3.39; P = 0.007). On the other hand, maternal TSH was not associated with cognitive delay.
2012	Lazarus	The CATS study was the first randomised controlled trial of an intervention in women found to have suboptimal thyroid function. Pregnant women at average 12 weeks gestation in South Wales and Turin were recruited (>21,000) and those with fT4 in the lowest 2.5% or TSH in the highest 2.5% centile were randomised to treatment with levothyroxine. The children underwent IQ assessments at age 3. Two further studies published ****
2016	Korevaar	Part of the Generation R studies, with 646 MRI scans available of children with known IQ and maternal thyroid function. At both high and low maternal thyroxine concentrations were associated with lower child IQ and lower grey matter and cortex volume, with a U shaped curve. The authors suggest an association between high maternal free thyroxine and lower child IQ suggests that levothyroxine therapy during pregnancy, which aims to achieve high-normal thyroid function, may carry a potential risk of adverse child neurodevelopment outcomes.
2017	Casey	Randomised control trial in the US, screening >90,000 pregnant women. “A total of 677 women with subclinical hypothyroidism underwent randomization at a mean of 16.7 weeks of gestation, and 526 with hypothyroxinemia at a mean of 17.8 weeks of gestation” to treatment with

		levothyroxine. No difference found in children's IQ at age 5 years old.
2018	Hales	Addressed some of the questions from CATS I. Confirmed did not find Haddow's findings of effect on IQ compared to normal controls.
2020	Hales	Behaviour Although CATS II did not find an association between maternal thyroid function and IQ, it did find a link between ADHD traits and maternal hyperthyroidism (unpublished data awaiting submission). The difference was seen in children of 'over-treated' mothers displayed significantly more ADHD symptoms and behavioural difficulties than those with normal-GTF. There was no overall association between SGTF and offspring ADHD, ASD or behaviour questionnaire scores.

7.2 PRISMA Checklist, 2020

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a literature review.	Page 47
ABSTRACT			
Abstract	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings. See the PRISMA 2020 for Abstracts checklist for the complete list.	NA – Included in abstract of thesis.
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge, i.e., what is already known about your topic.	Page 48
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 34, 48-49
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses with study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 50
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 50
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 49 refers to Appendix 7.3, page 178
Selection process	8	State the process for selecting studies (i.e., screening, eligibility). Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 49
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 51
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 51
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were	Page 53 (Figure 3.1)

Appendix

Section and Topic	Item #	Checklist item	Location where item is reported
		excluded.	
Study characteristics	17	Cite each included study and present its characteristics (e.g., study size, PICOS, follow-up period).	Pages 71-74 for summary table 3.2
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Pages 71-74, Included in table 3.2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Not applicable
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Pages 75- 86
	23b	Discuss any limitations of the evidence included in the review.	Page 86 – 87
	23c	Discuss any limitations of the review processes used.	Page 86
	23d	Discuss implications of the results for practice, policy, and future research.	Page 87
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Not registered (thesis review was only conducted by myself. For publication, should be done by 2 independent reviewers.
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	NA
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	In the acknowledgements of the thesis I have acknowledged the fellowship from the Royal College of Physicians that funded my research degree. No specific funding for the review.
Competing interests	26	Declare any competing interests of review authors.	NA
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Pages 178-180 for search and example of data collection form

Adapted From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71 *Adapted From:* Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71 The checklist was adapted for KIN 4400 Independent Research Study in Kinesiology at the University of Guelph-Humber. Last updated: Dec 9, 2021

For more information, visit: http://www.prisma-statement.org/

7.3 Systematic Review Search

Initial search, 2019: EMBASE, Medline and PsychInfo and Scopus.

1. magnetic resonance imaging/
2. neuroimaging/
3. mri.tw.
4. neuroimaging.tw.
5. neuro imaging.tw.
6. magnetic resonance.tw.
7. brain imaging.tw.
8. Diffusion Magnetic Resonance Imaging/
9. diffusion tensor.tw.
10. fmri.mp.
11. functional magnetic resonance.tw.
12. neuroanatomy.tw.
13. neuro anatomy.tw.
14. thyroid hormones/ or dextrothyroxine/ or diiodotyrosine/ or monoiodotyrosine/ or "thyroid (usp)"/ or thyronines/ or thyroxine/
15. Thyroid Gland/
16. Thyroid Diseases/
17. hypothyroid*.tw.
18. hyperthyroid*.tw.
19. thyroid dysfunction.tw.
20. (gestational adj3 thyroid*).tw.
21. (maternal adj3 thyroid*).tw.
22. maternal hypothyroxin*.tw.
23. maternal hyperthyroxin*.tw.
24. hyper thyroid*.tw.
25. hyper-thyroid*.tw.
26. hypo thyroid*.tw.
27. hypo-thyroid*.tw.
28. hypothyroxin*.tw.
29. Congenital Hypothyroidism/
30. cretinism.mp.
31. cretin*.tw.
32. (thyroid* adj3 fetal).tw.
33. (thyroid* adj3 fetus).tw.
34. (thyroid* adj3 foetal).tw.
35. (thyroid* adj3 foetus).tw.
36. congential hypothyroidism.tw.

37.	14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36
38.	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13
39.	37 and 38
40.	limit 39 to (english language and humans)

Updated, search undertaken 2021

1.	magnetic resonance imaging/
2.	neuroimaging/
3.	mri.tw.
4.	neuroimaging.tw.
5.	neuro imaging.tw.
6.	magnetic resonance.tw.
7.	brain imaging.tw.
8.	Diffusion Magnetic Resonance Imaging/
9.	diffusion tensor.tw.
10.	fmri.mp.
11.	functional magnetic resonance.tw.
12.	neuroanatomy.tw.
13.	neuro anatomy.tw.
14.	thyroid hormones/ or dextrothyroxine/ or diiodotyrosine/ or monoiodotyrosine/ or "thyroid (usp)"/ or thyronines/ or thyroxine/
15.	Thyroid Gland/
16.	Thyroid Diseases/
17.	hypothyroid*.tw.
18.	hyperthyroid*.tw.
19.	thyroid dysfunction.tw.
20.	(gestational adj3 thyroid*).tw.
21.	(maternal adj3 thyroid*).tw.
22.	maternal hypothyroxin*.tw.
23.	maternal hyperthyroxin*.tw.
24.	hyper thyroid*.tw.
25.	hyper-thyroid*.tw.
26.	hypo thyroid*.tw.
27.	hypo-thyroid*.tw.
28.	hypothyroxin*.tw.
29.	Congenital Hypothyroidism/
30.	cretinism.mp.
31.	cretin*.tw.
32.	(thyroid* adj3 fetal).tw.

33.	(thyroid* adj3 fetus).tw.
34.	(thyroid* adj3 foetal).tw.
35.	(thyroid* adj3 foetus).tw.
36.	congential hypothyroidism.tw.
37.	14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36
38.	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13
39.	37 and 38
40.	limit 39 to (english language and humans)
41.	limit 40 to yr="2019 -Current"

Example of data collection spreadsheet:

Author	Akinci	Hung	Ng	Scratch	Siragusa	~~Altiay~~	~~Alves~~
Year	2006	2018	2014	2014	1997	~~2006~~	~~1989~~
Title	Brain MR spectroscop	Gestation	Effect of t	Free Thyro	Brain mag	~~Tc-99m-HI~~	~~Changes i~~
Country	Turkey	Taiwan	UK	Australia	Italy		
Include	Yes	Yes	Yes	Yes		~~No~~	~~No~~
Type of scan	Proton MRS	dTI	dTi	Structural	1.5 structu	~~SPECT, CT or MRI onl~~	
Age when scanned	5-7days and 8 weeks	postmens	premmies	7	mean 22 d	~~1-15years~~	
Number scanned	8CH & 8 control fullte	81 total st	42 scanne	52 useable	11CHs, 22	~~30~~	
Groups?				Born unde	CHs, term	~~Down~~	
Other tests	Serum TFTs, urinary i	Tracts ana	T4 supple	neonatal f		~~SPECT, variety~~	
Findings:	CH neonates & their	No difference relate		higher NO	No differences		
Type of study	Case control	cohort	interventi	prospectiv	case control		~~Single cas~~
Comments	conclusion a leap- th	Can't make ANY judg		Looked at			
Main points	Difference in thalam	Gestation	Low fT4 at birth was		no morph	~~Looking at~~	~~Case repo~~

7.4 CATS III Study R code for T1 analysis

For link to the online R code, go to open source framework at :

DOI 10.17605/OSF.IO/PRKU8

```
##T1 analysis script for CATS study
#Carolyn McNabb and Anna Scholz 2023
#Is there a relationship between maternal thyroid function in pregnancy
and white matter microstructure?
#Four groups =
# N=20 healthy;
#N=20 untreated low thyroid;
#N=21 optimally treated low thyroid;
#N=19 over-treated low thyroid
#suboptimal gestational thyroid function (3/4) and normal thyroid
function (1/4)
#N=60 low thyroid function before 12 weeks pregnancy
#After 12 weeks, N=40 treated, N=20 untreated
#N=19 of the 40 treated were over-treated, N=21 were optimally treated
#At age 3 and 9 years
#No significant difference in IQ
#Treated group at age 9, higher rates of ADHD characteristics
#The over-treated group could explain this difference
######### key questions #################
#Can we show that overtreatment is suboptimal for neurodevelopment?
#Are there suggestions that treatment with thyroxine can be beneficial
if it's given at the optimal dose?
#T1 cortical and subcortical structures
#Total cortical grey and white matter volumes (parcellated using
Desikan-Killiany atlas)
#Surface area (white, grey, csf regions)
#Volumes in mm3 (and areas - but we won't look at area)
#Maternal Free T4 and TSH at 12 weeks (and for optimally and over-
treated at 20 and 30 weeks gestation)
################ research questions ###########
#grey matter volume across the four groups
#does overtreatment with thyroxine cause changes in volume vs optimal
treatment / normal levels
#N=19 overtreated vs N=20 healthy and N=21 optimally treated
#Is there an association between T4/TSH levels before 12 weeks and
cortical/subcortical volume?
#normal (N=20) vs low thyroid (N=60)
#add libraries
library(readODS)
library(psych)
library(vtable)
library(ggplot2)
library(emmeans)
library(rstatix)
#library(corrplot)
library(dplyr)
library(lme4)
library(lmerTest)
library(tidyr)
library(ppcor)
library(insight)
```

```
library(gt)
library(gridExtra)
# load demographic data
demographics <- read_ods('aparc_volume_left_Mar21_AS_allTFTs.ods',
sheet = 2)
#remove duplicate id column from demographics table
demographics <- demographics[, !duplicated(colnames(demographics))]
#copy the demographic dataframe so you can work on it
clean_demog <- demographics
#clean up total tanner score - first make numeric
clean_demog$`Total Tanner` <- as.numeric(clean_demog$`Total Tanner`)
#replace 0 with NA
clean_demog$`Total Tanner`[clean_demog$`Total Tanner` == 0] <- NA
#replace NAs in Total Tanner so that we can include these in the
analysis
#replace with median tanner score from the rest of the cohort - we
checked the
#age of the ppts and it is approximately the median age of the cohort
clean_demog$`Total Tanner`[is.na(clean_demog$`Total Tanner`)] <-
median(clean_demog$`Total Tanner`, na.rm=TRUE)
#make sex a factor
clean_demog$`Child Sex (1boy 2girl)` <- as.factor(clean_demog$`Child
Sex (1boy 2girl)`)
#add a column for effect coding of sex
clean_demog$sex <- ifelse(clean_demog$`Child Sex (1boy 2girl)` == 1,
0,1)
#make tsh and freet4 numeric
clean_demog$tsh <- as.numeric(clean_demog$tsh)
clean_demog$freet4 <- as.numeric(clean_demog$freet4)
#Now these have NAs so we need to remove these.. BUT, we will do this
individually later,
#so that we can include as much data as possible in each analysis
#name groups in the clean_demog dataframe
clean_demog$TreatmentGroup <- clean_demog$`GROUP (0 normal 1 treated 2
untreated 3 OT)`
clean_demog$TreatmentGroup[clean_demog$TreatmentGroup == "0"] <-
"Normal GTF"
clean_demog$TreatmentGroup[clean_demog$TreatmentGroup == "1"] <-
"Optimally treated SGTF"
clean_demog$TreatmentGroup[clean_demog$TreatmentGroup == "2"] <-
"Untreated SGTF"
clean_demog$TreatmentGroup[clean_demog$TreatmentGroup == "3"] <- "Over-
treated SGTF"
#Data for total volume - aseg
#load the volume data into R - call this aseg
aseg <- read_ods('aseg_volume_Mar21_AS_allTFTs.ods', sheet = 1,
row_names = F)
### Change CUBRIC IDs to CATS IDs ####
#first read in the cats lookup table
catsids <- read.table('CATS_ID_lookup.txt', header = T)
#add the CATS IDs to the aseg dataframe
aseg_ids <- merge(catsids, aseg, by.x = "CUBRIC_ID", by.y = "CUBRIC
ID")
#add the demographics data to the same dataframe
aseg_demog <- merge(clean_demog, aseg_ids, by.x = "CATS ID", by.y =
"CATS_ID")
############################### check assumptions ###################
```

```
#check assumptions for correlation analysis - both variables should
have normal distributions
num_data <- select_if(aseg_demog, is.numeric)
lshap <- lapply(num_data[c(2,4:6,60, 63:65,75)], shapiro.test)
lres <- sapply(lshap, `[`, c("statistic","p.value"))
non_normal <- colnames(lres)[lres["p.value",]<0.05]
normal <- colnames(lres)[lres["p.value",]>=0.05]
#check assumptions for ANOVA - should have normal distributions (which
we have as shown above) and
#homogeneity of variances
group_num_data <- cbind(aseg_demog$TreatmentGroup, num_data)
colnames(group_num_data)[1] <- c("TreatmentGroup")
bart_test <- lapply(group_num_data[c(3, 5:7, 61, 64:66, 76)],
function(x) bartlett.test(x ~ TreatmentGroup, group_num_data))
bart_sum <- sapply(bart_test, `[`, c("statistic","p.value"))
non_homog <- colnames(bart_sum)[bart_sum["p.value",]<0.05]
homog <- colnames(bart_sum)[bart_sum["p.value",]>=0.05]
#print results
print(normal)
print(homog)
print(non_normal)
print(non_homog)
###################### Summary statistics ############################
#Get all summary statisics for each of the groups:
describeBy(clean_demog, group = "GROUP (0 normal 1 treated 2 untreated
3 OT)")
#Table 1: Demographics
st(aseg_demog,group = "TreatmentGroup", var = c("tsh", "freet4",
                                                "Child Sex (1boy
2girl)",
                                                "Age at date of scan",
                                                "Total Tanner"),
   group.test = F, digits = 4, out = "viewer", title = "Table 1:
Participant demogrphics",
   add.median = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Normal GTF", "tsh"], na.rm
= T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Untreated SGTF", "tsh"],
na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Optimally treated SGTF",
"tsh"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Over-treated SGTF",
"tsh"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Normal GTF", "freet4"],
na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Untreated SGTF",
"freet4"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Optimally treated SGTF",
"freet4"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Over-treated SGTF",
"freet4"], na.rm = T)
#To get p values, run each of these tests using an anova or Kruskal-
Wallis (using rstatix)
kruskal_test(aseg_demog, tsh ~ TreatmentGroup) #p=.000000533
kruskal_test(aseg_demog, freet4 ~ TreatmentGroup) #p=.000192
kruskal_test(aseg_demog, `Child Sex (1boy 2girl)` ~ TreatmentGroup)
#p=ns
kruskal_test(aseg_demog, `Age at date of scan` ~ TreatmentGroup) #p=ns
```

```
kruskal_test(aseg_demog, `Total Tanner` ~ TreatmentGroup) #p=ns
#posthoc tests for tsh and freet4
pairwise.wilcox.test(aseg_demog$tsh, aseg_demog$TreatmentGroup,
p.adjust.method = "bonferroni",
                     paired = FALSE)
pairwise.wilcox.test(aseg_demog$freet4, aseg_demog$TreatmentGroup,
p.adjust.method = "bonferroni",
                     paired = FALSE)
############ Primary endpoint - Group analysis ################
## Kruskal-Wallis test by rank is a non-parametric alternative to one-
way ANOVA test,
#which extends the two-samples Wilcoxon test in the situation where
there are more than two groups.
#It's recommended when the assumptions of one-way ANOVA test are not
met.
#Table 2: Global measures of brain morphology
#produce a table with the summary stats for each group using vtable
st(aseg_demog,group = "TreatmentGroup", var = c("TotalGrayVol",
"CortexVol",

"CerebralWhiteMatterVol",
                                                  "SubCortGrayVol",

"EstimatedTotalIntraCranialVol"),
   group.test = F, out = "viewer", title = "Table 2: Global measures of
brain morphology")
#Note that for Table 2, you will want to report median and IQR for
SubCortGrayVol the data will be analysed using a non-parametric test
#To get p values, run each of these tests using an anova or Kruskal-
Wallis (using rstatix)
anova_test(aseg_demog, TotalGrayVol ~ TreatmentGroup)
anova_test(aseg_demog, CortexVol ~ TreatmentGroup)
anova_test(aseg_demog, CerebralWhiteMatterVol ~ TreatmentGroup)
kruskal_test(aseg_demog, SubCortGrayVol ~ TreatmentGroup) #note that
SubCortGrayVol demonstrates heteroscedasticity so we use a non-
parametric test
anova_test(aseg_demog, EstimatedTotalIntraCranialVol ~ TreatmentGroup)
#Include covariates in the models using a multiple linear regression
summary(lm(TotalGrayVol ~ TreatmentGroup + sex +
     `Age at date of scan` + `Total Tanner`, aseg_demog))
summary(lm(CortexVol ~ TreatmentGroup + sex +
             `Age at date of scan` + `Total Tanner`, aseg_demog))
summary(lm(CerebralWhiteMatterVol ~ TreatmentGroup + sex +
             `Age at date of scan` + `Total Tanner`, aseg_demog))
summary(lm(SubCortGrayVol ~ TreatmentGroup + sex +
             `Age at date of scan` + `Total Tanner`, aseg_demog))
summary(lm(EstimatedTotalIntraCranialVol ~ TreatmentGroup + sex +
             `Age at date of scan` + `Total Tanner`, aseg_demog))
#Make group a factor
clean_demog$TreatmentGroup <- as.factor(clean_demog$TreatmentGroup)
#test the effect of group*sex (interaction) using a factorial anova
df <- aseg_demog
df$sex <- as.factor(df$`Child Sex (1boy 2girl)`)
anova_test(df, TotalGrayVol ~ TreatmentGroup*sex) # no significant
interaction
#between group and sex - this means that the effect of group is not
different
#between boys and girls
```

```
anova_test(df, CortexVol ~ TreatmentGroup*sex) #again no interaction
anova_test(df, CerebralWhiteMatterVol ~ TreatmentGroup*sex) # again no
interaction
anova_test(df, SubCortGrayVol ~ TreatmentGroup*sex) # again no
interaction
anova_test(df, EstimatedTotalIntraCranialVol ~ TreatmentGroup*sex) #
again no interaction
########### plots for group effects ######################
#first we need to gather the data so that we have it in long format (so
it can be plotted nicely)
new_df <- aseg_demog[c("TreatmentGroup","TotalGrayVol", "CortexVol",
"CerebralWhiteMatterVol",  "SubCortGrayVol",
"EstimatedTotalIntraCranialVol")]
colnames(new_df) <- c("TreatmentGroup","Total Gray", "Cortex",
"Cerebral White",  "Subcortical Gray", "Total Intracranial")
long_groupdata <- gather(new_df,"Metric","Tissue Volume",2:6)
long_groupdata$volx105 <- long_groupdata$`Tissue Volume`*10e-5
long_groupdata$TreatmentGroup <- factor(long_groupdata$TreatmentGroup,
                                        levels=c("Normal GTF",
"Untreated SGTF",
                                                 "Optimally treated
SGTF",
                                                 "Over-treated SGTF"))
ggplot(long_groupdata, aes(x=TreatmentGroup, y=`volx105`)) +
geom_point() +
  geom_boxplot() + facet_grid(rows = vars(Metric), scales="free") +
theme_bw() +
  labs( x = "Treatment Group", y = "Tissue Volume (x10e5 mm3)")
########### Secondary endpoint - TSH and Free T4 ##############
#Table 3: Effect of TSH and FreeT4 on global measures of brain
morphology
############ 12 weeks gestation
################################################
#Free T4:
#First identify outlier in freet4 data
# Get the freet4 data not including the NAs
freet4_demog <- filter(aseg_demog, !is.na(freet4))
#get outliers in terms of freeT4
out <- boxplot.stats(freet4_demog$freet4)$out
boxplot(freet4_demog$freet4)
clean_freet4 <- freet4_demog
#get the correlation (kendall statistic) for the freet4 vs brain
morphology metrics
#first, make an empty list
correlations_freet4_12wk <- list()
boys_freet4 <- list()
girls_freet4 <- list()
#then loop through metrics and get full and partial correlations for
the data with and without outliers
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(clean_freet4[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- clean_freet4[-which(clean_freet4[[metric]] %in%
out),]
```

```
  } else {
    freet4_data <- clean_freet4
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4", "Age at date
of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_12wk[[metric]][["Partial"]][["no_freet4_or_metric_o
utlier"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_freet4_12wk[[metric]][["Full"]][["no_freet4_or_metric_outl
ier"]] <- cor.test(data_for_correlations$freet4,
as.numeric(data_for_correlations[[metric]]), method = "kendall")

  ###################### interactions between freet4 and sex
####################
  boys <- data_for_correlations[data_for_correlations$sex == 0,]
  boys <- boys[-5] #remove 5th column, which is sex
  boys_freet4[[metric]][["Partial"]][["no_freet4_or_metric_outlier"]]
<- pcor( boys, method = "kendall" )
  boys_freet4[[metric]][["Full"]][["no_freet4_or_metric_outlier"]] <-
cor.test(boys$freet4, as.numeric(boys[[metric]]), method = "pearson")

  girls <- data_for_correlations[data_for_correlations$sex == 1,]
  girls <- girls[-5] #remove 5th column, which is sex
  girls_freet4[[metric]][["Partial"]][["no_freet4_or_metric_outlier"]]
<- pcor( girls, method = "kendall" )
  girls_freet4[[metric]][["Full"]][["no_freet4_or_metric_outlier"]] <-
cor.test(girls$freet4, as.numeric(girls[[metric]]), method = "pearson")


#########################################################################
###
  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_demog[c(metric, "freet4", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_freet4_12wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  correlations_freet4_12wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$freet4,
as.numeric(data_for_correlations[[metric]]), method = "kendall")


  ###################### interactions between freet4 and sex
####################
  boys <- data_for_correlations[data_for_correlations$sex == 0,]
  boys <- boys[-5] #remove 5th column, which is sex
  boys_freet4[[metric]][["Partial"]][["all_data"]] <- pcor( boys,
method = "kendall" )
  boys_freet4[[metric]][["Full"]][["all_data"]] <-
cor.test(boys$freet4, as.numeric(boys[[metric]]), method = "pearson")

  girls <- data_for_correlations[data_for_correlations$sex == 1,]
```

```
  girls <- girls[-5] #remove 5th column, which is sex
  girls_freet4[[metric]][["Partial"]][["all_data"]] <- pcor( girls,
method = "kendall" )
  girls_freet4[[metric]][["Full"]][["all_data"]] <-
cor.test(girls$freet4, as.numeric(girls[[metric]]), method = "pearson")
}
#TSH:
# Get the tsh data not including the NAs
tsh_demog <- filter(aseg_demog, !is.na(tsh))
#Get outliers in terms of TSH
out <- boxplot.stats(tsh_demog$tsh)$out
boxplot(tsh_demog$tsh)
which(tsh_demog$tsh %in% out)
# remove outlier from tsh data and save as a different data_frame
clean_tsh <- tsh_demog[-which(tsh_demog$tsh %in% out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_tsh_12wk <- list()
boys_tsh <- list()
girls_tsh <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(clean_tsh[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- clean_tsh[-which(clean_tsh[[metric]] %in% out),]
  } else {
    tsh_data <- clean_tsh
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tsh", "Age at date of
scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_12wk[[metric]][["Partial"]][["no_tsh_or_metric_outlier
"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_tsh_12wk[[metric]][["Full"]][["no_tsh_or_metric_outlier"]]
<- cor.test(data_for_correlations$tsh,
as.numeric(data_for_correlations[[metric]]), method = "kendall")


  ###################### interactions between tsh and sex
####################
  boys <- data_for_correlations[data_for_correlations$sex == 0,]
  boys <- boys[-5] #remove 5th column, which is sex
  boys_tsh[[metric]][["Partial"]][["no_tsh_or_metric_outlier"]] <-
pcor( boys, method = "kendall" )
  boys_tsh[[metric]][["Full"]][["no_tsh_or_metric_outlier"]] <-
cor.test(boys$tsh, as.numeric(boys[[metric]]), method = "pearson")

  girls <- data_for_correlations[data_for_correlations$sex == 1,]
  girls <- girls[-5] #remove 5th column, which is sex
  girls_tsh[[metric]][["Partial"]][["no_tsh_or_metric_outlier"]] <-
pcor( girls, method = "kendall" )
```

```
  girls_tsh[[metric]][["Full"]][["no_tsh_or_metric_outlier"]] <-
cor.test(girls$tsh, as.numeric(girls[[metric]]), method = "pearson")


#########################################################################
####
  #data with only the tsh outlier and not the metric outlier
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- clean_tsh[c(metric, "tsh", "Age at date of
scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_tsh_12wk[[metric]][["Partial"]][["no_tsh_oulier"]] <-
pcor( data_for_correlations, method = "kendall" )
  correlations_tsh_12wk[[metric]][["Full"]][["no_tsh_outlier"]] <-
cor.test(data_for_correlations$tsh,
as.numeric(data_for_correlations[[metric]]), method = "kendall")


#########################################################################
####
  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_demog[c(metric, "tsh", "Age at date of
scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_tsh_12wk[[metric]][["Partial"]][["all_data"]] <- pcor(
data_for_correlations, method = "kendall" )
  correlations_tsh_12wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$tsh,
as.numeric(data_for_correlations[[metric]]), method = "kendall")


  ###################### interactions between tsh and sex
####################
  boys <- data_for_correlations[data_for_correlations$sex == 0,]
  boys <- boys[-5] #remove 5th column, which is sex
  boys_tsh[[metric]][["Partial"]][["all_data"]] <- pcor( boys, method =
"kendall" )
  boys_tsh[[metric]][["Full"]][["all_data"]] <- cor.test(boys$tsh,
as.numeric(boys[[metric]]), method = "pearson")
  girls <- data_for_correlations[data_for_correlations$sex == 1,]
  girls <- girls[-5] #remove 5th column, which is sex
  girls_tsh[[metric]][["Partial"]][["all_data"]] <- pcor( girls, method
= "kendall" )
  girls_tsh[[metric]][["Full"]][["all_data"]] <- cor.test(girls$tsh,
as.numeric(girls[[metric]]), method = "pearson")
}
################### 6 weeks after treatment initiation (~20 weeks
gestation) ####################
# Free T4
# Get the freet4 @ 20 weeks data not including the NAs
freet4_demogwk6 <- filter(aseg_demog, !is.na(freet4levelat6wk))
#get outliers in terms of freeT4
out <- boxplot.stats(freet4_demogwk6$freet4levelat6wk)$out
boxplot(freet4_demogwk6$freet4levelat6wk)
clean_freet4wk6 <- freet4_demogwk6
```

```
#create loop to run correlations and partial correlations for each
metric
correlations_freet4_20wk <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(clean_freet4wk6[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- clean_freet4wk6[-which(clean_freet4wk6[[metric]]
%in% out),]
  } else {
    freet4_data <- clean_freet4wk6
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat6wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_20wk[[metric]][["Partial"]][["no_freet4_or_metric_o
utlier"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_freet4_20wk[[metric]][["Full"]][["no_freet4_or_metric_outl
ier"]] <- cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_demogwk6[c(metric,
"freet4levelat6wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_freet4_20wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  correlations_freet4_20wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
# Get the tsh @ 20 weeks data not including the NAs
tsh_demogwk6 <- filter(aseg_demog, !is.na(tshlevelat6wk))
#change character NA strings to actual NAs
tsh_demogwk6$tshlevelat6wk <- as.numeric(tsh_demogwk6$tshlevelat6wk)
#remove NAs from data
tsh_demogwk6 <- filter(tsh_demogwk6, !is.na(tshlevelat6wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_demogwk6$tshlevelat6wk)$out
boxplot(tsh_demogwk6$tshlevelat6wk)
clean_tshwk6 <- tsh_demogwk6[-which(tsh_demogwk6$tshlevelat6wk %in%
out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_tsh_20wk <- list()
```

```
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(clean_tshwk6[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- clean_tshwk6[-which(clean_tshwk6[[metric]] %in% out),]
  } else {
    tsh_data <- clean_tshwk6
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tshlevelat6wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_20wk[[metric]][["Partial"]][["no_tsh_or_metric_outlier
"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_tsh_20wk[[metric]][["Full"]][["no_tsh_or_metric_outlier"]]
<- cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with only the tsh outlier and not the metric outlier
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- clean_tshwk6[c(metric, "tshlevelat6wk", "Age
at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_tsh_20wk[[metric]][["Partial"]][["no_tsh_oulier"]] <-
pcor( data_for_correlations, method = "kendall" )
  correlations_tsh_20wk[[metric]][["Full"]][["no_tsh_outlier"]] <-
cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_demogwk6[c(metric, "tshlevelat6wk", "Age
at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_tsh_20wk[[metric]][["Partial"]][["all_data"]] <- pcor(
data_for_correlations, method = "kendall" )
  correlations_tsh_20wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
################### 16 weeks after treatment initiation (~30 weeks
gestation) ######################
# Get the freet4 @ 30 weeks data not including the NAs
freet4_demogwk30 <- filter(aseg_demog, !is.na(freet4levelat30wk))
#change character NA strings to actual NAs
```

```
freet4_demogwk30$freet4levelat30wk <-
as.numeric(freet4_demogwk30$freet4levelat30wk)
#remove NAs from data
freet4_demogwk30 <- filter(freet4_demogwk30, !is.na(freet4levelat30wk))
#get outliers in terms of freeT4
out <- boxplot.stats(freet4_demogwk30$freet4levelat30wk)$out
# no outliers
boxplot(freet4_demogwk30$freet4levelat30wk)
clean_freet4wk30 <- freet4_demogwk30
#create loop to run correlations and partial correlations for each
metric
correlations_freet4_30wk <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(clean_freet4wk30[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- clean_freet4wk30[-which(clean_freet4wk30[[metric]]
%in% out),]
  } else {
    freet4_data <- clean_freet4wk30
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_30wk[[metric]][["Partial"]][["no_freet4_or_metric_o
utlier"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_freet4_30wk[[metric]][["Full"]][["no_freet4_or_metric_outl
ier"]] <- cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_demogwk30[c(metric,
"freet4levelat30wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_freet4_30wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  correlations_freet4_30wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
# Get the tsh @ 30 weeks data not including the NAs
tsh_demogwk30 <- filter(aseg_demog, !is.na(tshlevelat30wk))
#change character NA strings to actual NAs
tsh_demogwk30$tshlevelat30wk <-
as.numeric(tsh_demogwk30$tshlevelat30wk)
#remove NAs from data
```

```
tsh_demogwk30 <- filter(tsh_demogwk30, !is.na(tshlevelat30wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_demogwk30$tshlevelat30wk)$out
boxplot(tsh_demogwk30$tshlevelat30wk)
clean_tshwk30 <- tsh_demogwk30[-which(tsh_demogwk30$tshlevelat30wk %in%
out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_tsh_30wk <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(clean_tshwk30[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- clean_tshwk30[-which(clean_tshwk30[[metric]] %in%
out),]
  } else {
    tsh_data <- clean_tshwk30
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tshlevelat30wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_30wk[[metric]][["Partial"]][["no_tsh_or_metric_outlier
"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_tsh_30wk[[metric]][["Full"]][["no_tsh_or_metric_outlier"]]
<- cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with only the tsh outlier and not the metric outlier
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- clean_tshwk30[c(metric, "tshlevelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_tsh_30wk[[metric]][["Partial"]][["no_tsh_oulier"]] <-
pcor( data_for_correlations, method = "kendall" )
  correlations_tsh_30wk[[metric]][["Full"]][["no_tsh_outlier"]] <-
cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_demogwk30[c(metric, "tshlevelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  correlations_tsh_30wk[[metric]][["Partial"]][["all_data"]] <- pcor(
data_for_correlations, method = "kendall" )
  correlations_tsh_30wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$tshlevelat30wk,
```

```
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
#### WE ALSO WANTED TO LOOK AT THESE EFFECTS IN THE TWO TREATED GROUPS
SEPARATELY ####
# free T4 and tsh correlations across brain regions for treated groups
###################    Over-treated SGTF(~20 weeks gestation)
######################
# Free T4 for overtreated
# Get the freet4 @ 20 weeks data not including the NAs
freet4_week20_overtreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Over-treated SGTF",],
!is.na(freet4levelat6wk))
#get outliers in terms of freeT4
out <- boxplot.stats(freet4_week20_overtreated$freet4levelat6wk)$out
boxplot(freet4_week20_overtreated$freet4levelat6wk)
freet4_demogwk20_overtreated <- freet4_week20_overtreated[-
which(freet4_week20_overtreated$freet4levelat6wk %in% out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_freet4_20wk_overtreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(freet4_demogwk20_overtreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- freet4_demogwk20_overtreated[-
which(freet4_demogwk20_overtreated[[metric]] %in% out),]
  } else {
    freet4_data <- freet4_demogwk20_overtreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat6wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_20wk_overtreated[[metric]][["Partial"]][["no_freet4
_or_metric_outlier"]] <- pcor( data_for_correlations, method =
"kendall" )

correlations_freet4_20wk_overtreated[[metric]][["Full"]][["no_freet4_or
_metric_outlier"]] <- cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_week20_overtreated[c(metric,
"freet4levelat6wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_20wk_overtreated[[metric]][["Partial"]][["all_data"
]] <- pcor( data_for_correlations, method = "kendall" )
```

```
correlations_freet4_20wk_overtreated[[metric]][["Full"]][["all_data"]]
<- cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
# OPTIMALLY TREATED
freet4_week20_optimallytreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Optimally treated
SGTF",], !is.na(freet4levelat6wk))
#get outliers in terms of freeT4
out <-
boxplot.stats(freet4_week20_optimallytreated$freet4levelat6wk)$out
boxplot(freet4_week20_optimallytreated$freet4levelat6wk)
freet4_demogwk20_optimallytreated <- freet4_week20_optimallytreated
#create loop to run correlations and partial correlations for each
metric
correlations_freet4_20wk_optimallytreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(freet4_demogwk20_optimallytreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- freet4_demogwk20_optimallytreated[-
which(freet4_demogwk20_optimallytreated[[metric]] %in% out),]
  } else {
    freet4_data <- freet4_demogwk20_optimallytreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat6wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_20wk_optimallytreated[[metric]][["Partial"]][["no_f
reet4_or_metric_outlier"]] <- pcor( data_for_correlations, method =
"kendall" )

correlations_freet4_20wk_optimallytreated[[metric]][["Full"]][["no_free
t4_or_metric_outlier"]] <-
cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_week20_optimallytreated[c(metric,
"freet4levelat6wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_20wk_optimallytreated[[metric]][["Partial"]][["all_
data"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_freet4_20wk_optimallytreated[[metric]][["Full"]][["all_dat
```

```
a"]] <- cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
####################   Over-treated SGTF(~30 weeks gestation)
######################
# Free T4 for overtreated
# Get the freet4 @ 20 weeks data not including the NAs
aseg_demog$freet4levelat30wk <-
as.numeric(aseg_demog$freet4levelat30wk)
freet4_week30_overtreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Over-treated SGTF",],
!is.na(freet4levelat30wk))
#get outliers in terms of freeT4
out <- boxplot.stats(freet4_week30_overtreated$freet4levelat30wk)$out
boxplot(freet4_week30_overtreated$freet4levelat30wk)
freet4_demogwk30_overtreated <- freet4_week30_overtreated
#create loop to run correlations and partial correlations for each
metric
correlations_freet4_30wk_overtreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(freet4_demogwk30_overtreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- freet4_demogwk30_overtreated[-
which(freet4_demogwk30_overtreated[[metric]] %in% out),]
  } else {
    freet4_data <- freet4_demogwk30_overtreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_30wk_overtreated[[metric]][["Partial"]][["no_freet4
_or_metric_outlier"]] <- pcor( data_for_correlations, method =
"kendall" )

correlations_freet4_30wk_overtreated[[metric]][["Full"]][["no_freet4_or
_metric_outlier"]] <- cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_week30_overtreated[c(metric,
"freet4levelat30wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_30wk_overtreated[[metric]][["Partial"]][["all_data"
]] <- pcor( data_for_correlations, method = "kendall" )
```

```
correlations_freet4_30wk_overtreated[[metric]][["Full"]][["all_data"]]
<- cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
# OPTIMALLY TREATED
freet4_week30_optimallytreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Optimally treated
SGTF",], !is.na(freet4levelat30wk))
#get outliers in terms of freeT4
out <-
boxplot.stats(freet4_week30_optimallytreated$freet4levelat30wk)$out
boxplot(freet4_week30_optimallytreated$freet4levelat30wk)
freet4_demogwk30_optimallytreated <- freet4_week30_optimallytreated
#create loop to run correlations and partial correlations for each
metric
correlations_freet4_30wk_optimallytreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(freet4_demogwk30_optimallytreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- freet4_demogwk30_optimallytreated[-
which(freet4_demogwk30_optimallytreated[[metric]] %in% out),]
  } else {
    freet4_data <- freet4_demogwk30_optimallytreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_30wk_optimallytreated[[metric]][["Partial"]][["no_f
reet4_or_metric_outlier"]] <- pcor( data_for_correlations, method =
"kendall" )

correlations_freet4_30wk_optimallytreated[[metric]][["Full"]][["no_free
t4_or_metric_outlier"]] <-
cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_week30_optimallytreated[c(metric,
"freet4levelat30wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_freet4_30wk_optimallytreated[[metric]][["Partial"]][["all_
data"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_freet4_30wk_optimallytreated[[metric]][["Full"]][["all_dat
```

```
a"]] <- cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
################### TSH      Over-treated SGTF(~20 weeks gestation)
######################
#TSH for overtreated
# Get the TSH @ 20 weeks data not including the NAs
tsh_week20_overtreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Over-treated SGTF",],
!is.na(tshlevelat6wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_week20_overtreated$tshlevelat6wk)$out
boxplot(tsh_week20_overtreated$tshlevelat6wk)
tsh_demogwk20_overtreated <- tsh_week20_overtreated[-
which(tsh_week20_overtreated$tshlevelat6wk %in% out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_tsh_20wk_overtreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(tsh_demogwk20_overtreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- tsh_demogwk20_overtreated[-
which(tsh_demogwk20_overtreated[[metric]] %in% out),]
  } else {
    tsh_data <- tsh_demogwk20_overtreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tshlevelat6wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_20wk_overtreated[[metric]][["Partial"]][["no_tsh_or_me
tric_outlier"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_tsh_20wk_overtreated[[metric]][["Full"]][["no_tsh_or_metri
c_outlier"]] <- cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_week20_overtreated[c(metric,
"tshlevelat6wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_20wk_overtreated[[metric]][["Partial"]][["all_data"]]
<- pcor( data_for_correlations, method = "kendall" )
  correlations_tsh_20wk_overtreated[[metric]][["Full"]][["all_data"]]
<- cor.test(data_for_correlations$tshlevelat6wk,
```

```
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
# OPTIMALLY TREATED
tsh_week20_optimallytreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Optimally treated
SGTF",], !is.na(tshlevelat6wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_week20_optimallytreated$tshlevelat6wk)$out
boxplot(tsh_week20_optimallytreated$tshlevelat6wk)
tsh_demogwk20_optimallytreated <- tsh_week20_optimallytreated[-
which(tsh_week20_optimallytreated$tshlevelat6wk %in% out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_tsh_20wk_optimallytreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(tsh_demogwk20_optimallytreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- tsh_demogwk20_optimallytreated[-
which(tsh_demogwk20_optimallytreated[[metric]] %in% out),]
  } else {
    tsh_data <- tsh_demogwk20_optimallytreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tshlevelat6wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_20wk_optimallytreated[[metric]][["Partial"]][["no_tsh_
or_metric_outlier"]] <- pcor( data_for_correlations, method = "kendall"
)

correlations_tsh_20wk_optimallytreated[[metric]][["Full"]][["no_tsh_or_
metric_outlier"]] <- cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_week20_optimallytreated[c(metric,
"tshlevelat6wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_20wk_optimallytreated[[metric]][["Partial"]][["all_dat
a"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_tsh_20wk_optimallytreated[[metric]][["Full"]][["all_data"]
] <- cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)
```

```
}
####################   Over-treated SGTF(~30 weeks gestation)
######################
# Free T4 for overtreated
# Get the tsh @ 20 weeks data not including the NAs
aseg_demog$tshlevelat30wk <- as.numeric(aseg_demog$tshlevelat30wk)
tsh_week30_overtreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Over-treated SGTF",],
!is.na(tshlevelat30wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_week30_overtreated$tshlevelat30wk)$out
boxplot(tsh_week30_overtreated$tshlevelat30wk)
tsh_demogwk30_overtreated <- tsh_week30_overtreated[-
which(tsh_week30_overtreated$tshlevelat30wk %in% out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_tsh_30wk_overtreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(tsh_demogwk30_overtreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- tsh_demogwk30_overtreated[-
which(tsh_demogwk30_overtreated[[metric]] %in% out),]
  } else {
    tsh_data <- tsh_demogwk30_overtreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tshlevelat30wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_30wk_overtreated[[metric]][["Partial"]][["no_tsh_or_me
tric_outlier"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_tsh_30wk_overtreated[[metric]][["Full"]][["no_tsh_or_metri
c_outlier"]] <- cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_week30_overtreated[c(metric,
"tshlevelat30wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_30wk_overtreated[[metric]][["Partial"]][["all_data"]]
<- pcor( data_for_correlations, method = "kendall" )
  correlations_tsh_30wk_overtreated[[metric]][["Full"]][["all_data"]]
<- cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
```

```
# OPTIMALLY TREATED
tsh_week30_optimallytreated <-
filter(aseg_demog[aseg_demog$TreatmentGroup=="Optimally treated
SGTF",], !is.na(tshlevelat30wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_week30_optimallytreated$tshlevelat30wk)$out
boxplot(tsh_week30_optimallytreated$tshlevelat30wk)
tsh_demogwk30_optimallytreated <- tsh_week30_optimallytreated[-
which(tsh_week30_optimallytreated$tshlevelat30wk %in% out), ]
#create loop to run correlations and partial correlations for each
metric
correlations_tsh_30wk_optimallytreated <- list()
for (metric in c("TotalGrayVol", "CortexVol", "CerebralWhiteMatterVol",
"SubCortGrayVol", "EstimatedTotalIntraCranialVol")){
  #identify outliers for the metric
  out <- boxplot.stats(tsh_demogwk30_optimallytreated[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- tsh_demogwk30_optimallytreated[-
which(tsh_demogwk30_optimallytreated[[metric]] %in% out),]
  } else {
    tsh_data <- tsh_demogwk30_optimallytreated
  }
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tshlevelat30wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_30wk_optimallytreated[[metric]][["Partial"]][["no_tsh_
or_metric_outlier"]] <- pcor( data_for_correlations, method = "kendall"
)

correlations_tsh_30wk_optimallytreated[[metric]][["Full"]][["no_tsh_or_
metric_outlier"]] <- cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_week30_optimallytreated[c(metric,
"tshlevelat30wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

correlations_tsh_30wk_optimallytreated[[metric]][["Partial"]][["all_dat
a"]] <- pcor( data_for_correlations, method = "kendall" )

correlations_tsh_30wk_optimallytreated[[metric]][["Full"]][["all_data"]
] <- cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
########### EXPLORATORY ANALYSIS ############################
######## supplementary results at week 12 (before treatment initiation)
###########
```

```
#Free T4:
#remove hypointensity and 5th ventricle data from aseg_demog and save
as supp_data
supp_data <- aseg_demog[-c(54, 56:60)]
# Get the freet4 @ 20 weeks data not including the NAs
freet4_demog <- filter(supp_data, !is.na(freet4))
#change character NA strings to actual NAs
freet4_demog$freet4 <- as.numeric(freet4_demog$freet4)
#remove NAs from data
freet4_demog <- filter(freet4_demog, !is.na(freet4))
#get outliers in terms of freet4
out <- boxplot.stats(freet4_demog$freet4)$out
boxplot(freet4_demog$freet4)
clean_freet4 <- freet4_demog
#create loop to run correlations and partial correlations for each
metric
supp_correlations_freet4_12wk <- list()
for (metric in colnames(supp_data)[22:81]){
  #identify outliers for the metric
  out <- boxplot.stats(clean_freet4[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- clean_freet4[-which(clean_freet4[[metric]] %in%
out),]
  } else {
    freet4_data <- clean_freet4
  }

  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4", "Age at date
of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

supp_correlations_freet4_12wk[[metric]][["Partial"]][["no_freet4_or_met
ric_outlier"]] <- pcor( data_for_correlations, method = "kendall" )

supp_correlations_freet4_12wk[[metric]][["Full"]][["no_freet4_or_metric
_outlier"]] <- cor.test(data_for_correlations$freet4,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_demog[c(metric, "freet4", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_freet4_12wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  supp_correlations_freet4_12wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$freet4,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
#TSH:
```

```
#remove hypointensity and 5th ventricle data from aseg_demog and save
as supp_data
supp_data <- aseg_demog[-c(54, 56:60)]
# Get the tsh @ 20 weeks data not including the NAs
tsh_demog <- filter(supp_data, !is.na(tsh))
#change character NA strings to actual NAs
tsh_demog$tsh <- as.numeric(tsh_demog$tsh)
#remove NAs from data
tsh_demog <- filter(tsh_demog, !is.na(tsh))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_demog$tsh)$out
boxplot(tsh_demog$tsh)
clean_tsh <- tsh_demog[-which(tsh_demog$tsh %in% out), ]
#create loop to run correlations and partial correlations for each
metric
supp_correlations_tsh_12wk <- list()
for (metric in colnames(supp_data)[22:81]){
  #identify outliers for the metric
  out <- boxplot.stats(clean_tsh[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- clean_tsh[-which(clean_tsh[[metric]] %in% out),]
  } else {
    tsh_data <- clean_tsh
  }

  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tsh", "Age at date of
scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

supp_correlations_tsh_12wk[[metric]][["Partial"]][["no_tsh_or_metric_ou
tlier"]] <- pcor( data_for_correlations, method = "kendall" )

supp_correlations_tsh_12wk[[metric]][["Full"]][["no_tsh_or_metric_outli
er"]] <- cor.test(data_for_correlations$tsh,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with only the tsh outlier and not the metric outlier
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- clean_tsh[c(metric, "tsh", "Age at date of
scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_tsh_12wk[[metric]][["Partial"]][["no_tsh_oulier"]]
<- pcor( data_for_correlations, method = "kendall" )
  supp_correlations_tsh_12wk[[metric]][["Full"]][["no_tsh_outlier"]] <-
cor.test(data_for_correlations$tsh,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
```

```
  data_for_correlations <- tsh_demog[c(metric, "tsh", "Age at date of
scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_tsh_12wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  supp_correlations_tsh_12wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$tsh,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
######## supplementary results at week 20 (6 weeks after treatment
initiation) ###########
# Free T4:
supp_data <- aseg_demog[-c(54, 56:60)]
# Get the freet4 @ 20 weeks data not including the NAs
freet4_demogwk20 <- filter(supp_data, !is.na(freet4levelat6wk))
#change character NA strings to actual NAs
freet4_demogwk20$freet4levelat20wk <-
as.numeric(freet4_demogwk20$freet4levelat6wk)
#remove NAs from data
freet4_demogwk20 <- filter(freet4_demogwk20, !is.na(freet4levelat6wk))
#get outliers in terms of freet4
out <- boxplot.stats(freet4_demogwk20$freet4levelat6wk)$out
boxplot(freet4_demogwk20$freet4levelat6wk)
clean_freet4wk20 <- freet4_demogwk20
#create loop to run correlations and partial correlations for each
metric
supp_correlations_freet4_20wk <- list()
for (metric in colnames(supp_data)[22:81]){
  #identify outliers for the metric
  out <- boxplot.stats(clean_freet4wk20[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- clean_freet4wk20[-which(clean_freet4wk20[[metric]]
%in% out),]
  } else {
    freet4_data <- clean_freet4wk20
  }

  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat6wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

supp_correlations_freet4_20wk[[metric]][["Partial"]][["no_freet4_or_met
ric_outlier"]] <- pcor( data_for_correlations, method = "kendall" )

supp_correlations_freet4_20wk[[metric]][["Full"]][["no_freet4_or_metric
_outlier"]] <- cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
```

```
  data_for_correlations <- freet4_demogwk20[c(metric,
"freet4levelat6wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_freet4_20wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  supp_correlations_freet4_20wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$freet4levelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
# TSH:
supp_data <- aseg_demog[-c(54, 56:60)]
# Get the freet4 @ 20 weeks data not including the NAs
tsh_demogwk20 <- filter(supp_data, !is.na(tshlevelat6wk))
#change character NA strings to actual NAs
tsh_demogwk20$tshlevelat20wk <- as.numeric(tsh_demogwk20$tshlevelat6wk)
#remove NAs from data
tsh_demogwk20 <- filter(tsh_demogwk20, !is.na(tshlevelat6wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_demogwk20$tshlevelat6wk)$out
boxplot(tsh_demogwk20$tshlevelat6wk)
clean_tshwk20 <- tsh_demogwk20[-which(tsh_demogwk20$tshlevelat6wk %in%
out), ]
#create loop to run correlations and partial correlations for each
metric
supp_correlations_tsh_20wk <- list()
for (metric in colnames(supp_data)[22:81]){
  #identify outliers for the metric
  out <- boxplot.stats(clean_tshwk20[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- clean_tshwk20[-which(clean_tshwk20[[metric]] %in%
out),]
  } else {
    tsh_data <- clean_tshwk20
  }

  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_data[c(metric, "tshlevelat6wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

supp_correlations_tsh_20wk[[metric]][["Partial"]][["no_tsh_or_metric_ou
tlier"]] <- pcor( data_for_correlations, method = "kendall" )

supp_correlations_tsh_20wk[[metric]][["Full"]][["no_tsh_or_metric_outli
er"]] <- cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with only the tsh outlier and not the metric outlier
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- clean_tshwk20[c(metric, "tshlevelat6wk",
"Age at date of scan", "Total Tanner", "sex"  )]
```

```
  #run partial correlation on these data
  supp_correlations_tsh_20wk[[metric]][["Partial"]][["no_tsh_oulier"]]
<- pcor( data_for_correlations, method = "kendall" )
  supp_correlations_tsh_20wk[[metric]][["Full"]][["no_tsh_outlier"]] <-
cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_demogwk20[c(metric, "tshlevelat6wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_tsh_20wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  supp_correlations_tsh_20wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$tshlevelat6wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
############ supplementary results at week 30 (16 weeks after treatment
initiation) #########
#Free T4
#remove hypointensity and 5th ventricle data from aseg_demog and save
as supp_data
supp_data <- aseg_demog[-c(54, 56:60)]
# Get the freet4 @ 20 weeks data not including the NAs
freet4_demogwk30 <- filter(supp_data, !is.na(freet4levelat30wk))
#change character NA strings to actual NAs
freet4_demogwk30$freet4levelat30wk <-
as.numeric(freet4_demogwk30$freet4levelat30wk)
#remove NAs from data
freet4_demogwk30 <- filter(freet4_demogwk30, !is.na(freet4levelat30wk))
#get outliers in terms of freet4
out <- boxplot.stats(freet4_demogwk30$freet4levelat30wk)$out
boxplot(freet4_demogwk30$freet4levelat30wk)
clean_freet4wk30 <- freet4_demogwk30
#create loop to run correlations and partial correlations for each
metric
supp_correlations_freet4_30wk <- list()
for (metric in colnames(supp_data)[22:81]){
  #identify outliers for the metric
  out <- boxplot.stats(clean_freet4wk30[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    freet4_data <- clean_freet4wk30[-which(clean_freet4wk30[[metric]]
%in% out),]
  } else {
    freet4_data <- clean_freet4wk30
  }

  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_data[c(metric, "freet4levelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
```

```
  #run partial correlation on these data

supp_correlations_freet4_30wk[[metric]][["Partial"]][["no_freet4_or_met
ric_outlier"]] <- pcor( data_for_correlations, method = "kendall" )

supp_correlations_freet4_30wk[[metric]][["Full"]][["no_freet4_or_metric
_outlier"]] <- cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- freet4_demogwk30[c(metric,
"freet4levelat30wk", "Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_freet4_30wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  supp_correlations_freet4_30wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$freet4levelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
#TSH:
#remove hypointensity and 5th ventricle data from aseg_demog and save
as supp_data
supp_data <- aseg_demog[-c(54, 56:60)]
# Get the tsh @ 20 weeks data not including the NAs
tsh_demogwk30 <- filter(supp_data, !is.na(tshlevelat30wk))
#change character NA strings to actual NAs
tsh_demogwk30$tshlevelat30wk <-
as.numeric(tsh_demogwk30$tshlevelat30wk)
#remove NAs from data
tsh_demogwk30 <- filter(tsh_demogwk30, !is.na(tshlevelat30wk))
#get outliers in terms of tsh
out <- boxplot.stats(tsh_demogwk30$tshlevelat30wk)$out
boxplot(tsh_demogwk30$tshlevelat30wk)
clean_tshwk30 <- tsh_demogwk30[-which(tsh_demogwk30$tshlevelat30wk %in%
out), ]
#create loop to run correlations and partial correlations for each
metric
supp_correlations_tsh_30wk <- list()
for (metric in colnames(supp_data)[22:81]){
  #identify outliers for the metric
  out <- boxplot.stats(clean_tshwk30[[metric]])$out
  #if outliers exist, remove from the data, otherwise just copy the
data to the new variable
  if (length(out) > 0){
    tsh_data <- clean_tshwk30[-which(clean_tshwk30[[metric]] %in%
out),]
  } else {
    tsh_data <- clean_tshwk30
  }

  #subset the data to just include the variables of interest that you
want to correlate
```

```
  data_for_correlations <- tsh_data[c(metric, "tshlevelat30wk", "Age at
date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data

supp_correlations_tsh_30wk[[metric]][["Partial"]][["no_tsh_or_metric_ou
tlier"]] <- pcor( data_for_correlations, method = "kendall" )

supp_correlations_tsh_30wk[[metric]][["Full"]][["no_tsh_or_metric_outli
er"]] <- cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with only the tsh outlier and not the metric outlier
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- clean_tshwk30[c(metric, "tshlevelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_tsh_30wk[[metric]][["Partial"]][["no_tsh_oulier"]]
<- pcor( data_for_correlations, method = "kendall" )
  supp_correlations_tsh_30wk[[metric]][["Full"]][["no_tsh_outlier"]] <-
cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

  #data with noone excluded - even outliers
  #subset the data to just include the variables of interest that you
want to correlate
  data_for_correlations <- tsh_demogwk30[c(metric, "tshlevelat30wk",
"Age at date of scan", "Total Tanner", "sex"  )]
  #run partial correlation on these data
  supp_correlations_tsh_30wk[[metric]][["Partial"]][["all_data"]] <-
pcor( data_for_correlations, method = "kendall" )
  supp_correlations_tsh_30wk[[metric]][["Full"]][["all_data"]] <-
cor.test(data_for_correlations$tshlevelat30wk,
as.numeric(data_for_correlations[[metric]]), method = "kendall", exact
= FALSE)

}
################## extract correlation and p values for supplementary
materials ##############
#FREE T4 TABLE
partialestimates <- list()
fullestimates <- list()
partialps <- list()
fullps <- list()
for (metric in colnames(supp_data)[22:81]){
  partialestimates[[metric]] <-
supp_correlations_freet4_12wk[[metric]][["Partial"]][["no_freet4_or_met
ric_outlier"]][["estimate"]][2]
  fullestimates[[metric]] <-
supp_correlations_freet4_12wk[[metric]][["Full"]][["no_freet4_or_metric
_outlier"]][["estimate"]]["tau"]
  partialps[[metric]]<-
supp_correlations_freet4_12wk[[metric]][["Partial"]][["no_freet4_or_met
ric_outlier"]][["p.value"]][2]
```

```
  fullps[[metric]] <-
supp_correlations_freet4_12wk[[metric]][["Full"]][["no_freet4_or_metric
_outlier"]][["p.value"]]

}
supp_partial_freet4 <- cbind(colnames(supp_data)[22:81],
as.data.frame(unlist(partialestimates)),as.data.frame(unlist(partialps)
))
supp_full_freet4 <- cbind(colnames(supp_data)[22:81],
as.data.frame(unlist(fullestimates)),as.data.frame(unlist(fullps)))
colnames(supp_partial_freet4) <- c("Region", "Partial Correlation
Estimate", "Partial Correlation P value")
colnames(supp_full_freet4) <- c("Region", "Full Correlation Estimate",
"Full Correlation P value")
all_supp_freet4 <- cbind(supp_full_freet4[c("Region", "Full Correlation
Estimate", "Full Correlation P value")],
                         supp_partial_freet4[c("Partial Correlation
Estimate", "Partial Correlation P value")])
rownames(all_supp_freet4) <- NULL
export_table(all_supp_freet4, format="html", title = "Region-specific
effects for Free T4", digits = 3)
###################
#TSH TABLE
partialestimates <- list()
fullestimates <- list()
partialps <- list()
fullps <- list()
for (metric in colnames(supp_data)[22:81]){
  partialestimates[[metric]] <-
supp_correlations_tsh_12wk[[metric]][["Partial"]][["no_tsh_or_metric_ou
tlier"]][["estimate"]][2]
  fullestimates[[metric]] <-
supp_correlations_tsh_12wk[[metric]][["Full"]][["no_tsh_or_metric_outli
er"]][["estimate"]]["tau"]

  partialps[[metric]]<-
supp_correlations_tsh_12wk[[metric]][["Partial"]][["no_tsh_or_metric_ou
tlier"]][["p.value"]][2]
  fullps[[metric]] <-
supp_correlations_tsh_12wk[[metric]][["Full"]][["no_tsh_or_metric_outli
er"]][["p.value"]]

}
supp_partial_tsh <- cbind(colnames(supp_data)[22:81],
as.data.frame(unlist(partialestimates)),as.data.frame(unlist(partialps)
))
supp_full_tsh <- cbind(colnames(supp_data)[22:81],
as.data.frame(unlist(fullestimates)),as.data.frame(unlist(fullps)))
colnames(supp_partial_tsh) <- c("Region", "Partial Correlation
Estimate", "Partial Correlation P value")
colnames(supp_full_tsh) <- c("Region", "Full Correlation Estimate",
"Full Correlation P value")
all_supp_tsh <- cbind(supp_full_tsh[c("Region", "Full Correlation
Estimate", "Full Correlation P value")],
                         supp_partial_tsh[c("Partial Correlation
Estimate", "Partial Correlation P value")])
rownames(all_supp_tsh) <- NULL
```

```
export_table(all_supp_tsh, format="html", title = "Region-specific
effects for TSH", digits = 3, zap_small=FALSE)
######### Calculate Bonferroni correction (alpha value) ###########
new_alpha <- .05/nrow(all_supp_freet4)
all_supp_freet4$Region[which(all_supp_freet4$`Full Correlation P
value`< new_alpha)]
all_supp_freet4$Region[which(all_supp_freet4$`Partial Correlation P
value` < new_alpha)]
all_supp_tsh$Region[which(all_supp_tsh$`Full Correlation P value` <
new_alpha)]
all_supp_tsh$Region[which(all_supp_tsh$`Partial Correlation P value` <
new_alpha)]
##For 20 and 30 week demographic table##
#round(aseg_demog$tshlevelat30wk, 3)
#make 30 wk tsh and freet4 numeric, as too big a number
aseg_demog$tsh30wk <- as.numeric(aseg_demog$tshlevelat30wk)
aseg_demog$freet430wk <- as.numeric(aseg_demog$freet4levelat30wk)
#Now these have NAs so we need to remove these.. BUT, we will do this
individually later,
#so that we can include as much data as possible in each analysis
#Table 1b: Demographics
st(aseg_demog,group = "TreatmentGroup", var = c("tsh", "freet4",
"tshlevelat6wk", "tsh30wk", "freet4levelat6wk", "freet430wk",
                                                "Child Sex (1boy
2girl)",
                                                "Age at date of scan",
                                                "Total Tanner"),
   group.test = F, digits = 4, out = "viewer", title = "Table 1b:
Participant demographics",
   add.median = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Normal GTF", "tsh"], na.rm
= T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Untreated SGTF", "tsh"],
na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Optimally treated SGTF",
"tshlevelat6wk"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Over-treated SGTF",
"tshlevelat6wk"], na.rm = T)
#to get the 1st and 3rd quartiles, use summary function
summary(aseg_demog[aseg_demog$TreatmentGroup == "Optimally treated
SGTF", "tsh"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Normal GTF", "freet4"],
na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Untreated SGTF",
"freet4"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Optimally treated SGTF",
"freet4"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Over-treated SGTF",
"freet4"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Normal GTF",
"tshlevelat6wk"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Untreated SGTF",
"tshlevelat6wk"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Optimally treated SGTF",
"tshlevelat6wk"], na.rm = T)
IQR(aseg_demog[aseg_demog$TreatmentGroup == "Over-treated SGTF",
"tshlevelat6wk"], na.rm = T)
```

```
#To get p values, run each of these tests using an anova or Kruskal-
Wallis (using rstatix)
kruskal_test(aseg_demog, tsh ~ TreatmentGroup) #p=.000000533
kruskal_test(aseg_demog, freet4 ~ TreatmentGroup) #p=.000192
kruskal_test(aseg_demog, `Child Sex (1boy 2girl)` ~ TreatmentGroup)
#p=ns
kruskal_test(aseg_demog, `Age at date of scan` ~ TreatmentGroup) #p=ns
kruskal_test(aseg_demog, `Total Tanner` ~ TreatmentGroup) #p=ns
#posthoc tests for tsh and freet4
pairwise.wilcox.test(aseg_demog$tsh, aseg_demog$TreatmentGroup,
p.adjust.method = "bonferroni",
                     paired = FALSE)
pairwise.wilcox.test(aseg_demog$freet4, aseg_demog$TreatmentGroup,
p.adjust.method = "bonferroni",
                     paired = FALSE)
#week 20 and 30 data for table 1
#freet4
week20freet4 <- aseg_demog[!is.na(aseg_demog$freet4levelat6wk) , ]
wilcox.test(week20freet4[week20freet4$TreatmentGroup == "Optimally
treated SGTF", "freet4levelat6wk"],
       week20freet4[week20freet4$TreatmentGroup == "Over-treated SGTF",
"freet4levelat6wk"])
week30freet4 <- aseg_demog[!is.na(aseg_demog$freet4levelat30wk) , ]
wilcox.test(week30freet4[week30freet4$TreatmentGroup == "Optimally
treated SGTF", "freet4levelat30wk"],
       week30freet4[week30freet4$TreatmentGroup == "Over-treated SGTF",
"freet4levelat30wk"])
#tsh
week20tsh <- aseg_demog[!is.na(aseg_demog$tshlevelat6wk) , ]
wilcox.test(week20tsh[week20tsh$TreatmentGroup == "Optimally treated
SGTF", "tshlevelat6wk"],
       week20tsh[week20tsh$TreatmentGroup == "Over-treated SGTF",
"tshlevelat6wk"])
week30tsh <- aseg_demog[!is.na(aseg_demog$tshlevelat30wk) , ]
wilcox.test(week30tsh[week30tsh$TreatmentGroup == "Optimally treated
SGTF", "tshlevelat30wk"],
       week30tsh[week30tsh$TreatmentGroup == "Over-treated SGTF",
"tshlevelat30wk"])
#Anna trying to anova with IQ data. IQ_Demo_CATS
#name groups in the clean_demog dataframe
library(psych)
# load IQ data
IQ_Demo_CATS <- read.csv('IQ_Demo_CATS.csv')
IQ_Demo_CATS$Group[IQ_Demo_CATS$Group == "0"] <- "Normal GTF"
IQ_Demo_CATS$Group[IQ_Demo_CATS$Group == "1"] <- "Optimally treated
SGTF"
IQ_Demo_CATS$Group[IQ_Demo_CATS$Group == "2"] <- "Untreated SGTF"
IQ_Demo_CATS$Group[IQ_Demo_CATS$Group == "3"] <- "Over-treated SGTF"
#see if there is a difference in IQ between groups
describeBy(IQ_Demo_CATS$VCIQ, group = IQ_Demo_CATS$Group)
kruskal_test(IQ_Demo_CATS, VCIQ ~ Group)
clean_IQ <- IQ_Demo_CATS
#clean up total tanner score - first make numeric
clean_IQ$'FSIQ' <- as.numeric(clean_IQ$`FSIQ`)
#replace 0 with NA
clean_IQ$'FSIQ'[clean_IQ$`FSIQ` == 0] <- NA
num_data <- select_if(clean_IQ, is.numeric)
lshap <- lapply(num_data[c("FSIQ", "PSIQ")], shapiro.test)
```

```
lres <- sapply(lshap, `[`, c("statistic","p.value"))
non_normal <- colnames(lres)[lres["p.value",]<0.05]
normal <- colnames(lres)[lres["p.value",]>=0.05]
```

Appendix

Pre-processing steps

Pre-processing steps

The following applies when running individual subjects

Process the T1 image

cd to folder of the T1w scan

run dcm2nii *dcm

copy the .nii file and rename to T1_mprage.nii.gz - e.g. cp *.nii* ../../../T1_mprage.nii.gz (the cp ../../../ moves the file back a few folders where it will be easier to work with)

cd to the folder when the T1 file now is by running cd ../../../

Then run the following commands which will produce a dowsampled, skullstripped version of the T1w image

To do this it's best to use sinteractive as it is quite resource intensive

N4BIASFIELDCORRECTION

N4BiasFieldCorrection -v -d 3 -i T1_mprage.nii.gz -s 2 -c [100x100x100x100,0.0000000001] -o T1_mprage_n4.nii.gz

Skullstripping

bet T1_mprage_n4.nii.gz original_bet_T1.nii.gz -v -R -f 0.40 -g 0

Downsample data

/cubric/software/afni/3dresample -rmode Li -dxyz 1.5 1.5 1.5 -prefix downsampled_bet_T1.nii -insert original_bet_T1.nii

Get the Diffusion scans

Whilst this is running you can then get the other diffusion files you need - cd into the directory with the diffusion images (e.g. '4_diff 2Shell (30x1200,60x2400) Mono DFC_DFC_MIX') . Then run dcm2nii * dcm

Run gunzip on the nifti image to unzip the file

Then copy the .nii file to the folder when the processed T1 image is (e.g. cp filename.nii ../../../DWI.nii)

copy the bval file (e.g. cp filename.bval ../../../bval.bval)

copy the bvec file (e.g. cp filename.bvec ../../../bvec.bvec)

The next step is then to run the diffusion processing pipeline in matlab. The xml file is called pipeline_MTCSD.xml

Output stored in each subjects' folder in a folder called Diffusion_processing

MRI scanning protocol for the CATS III study

7.5 MRI scanning protocol

Icon below provides a link to the pdf document containing the whole MRI scanning protocol for CATS III study, including T1, DTI and qMT protocols.

CATS_MRI_protocol.pdf

7.6 CATS III study information for participants, consent and assent forms.

- Information pack sent to parents and children
- Consent and assent forms

Title of study: Suboptimal maternal thyroid function as a window to mechanisms of childhood brain development and function (CATS III)

Introduction

We would like to invite you and your child to take part in our research study. Before you decide whether you would like to participate, it is important that you understand why the research is being done and what it will involve for you.

Please take time to read the information outlined in this document carefully. Part 1 of this document tells you the purpose of this study and what will happen to you if you choose to participate. Part 2 gives you more detailed information about the conduct of the study. You may wish to discuss this information with others.

If there is anything that is unclear, or you require more information, you can get in touch with the research team.

Thank you for reading this document. CATS III; Mother information sheet. Version 3 Oct 2018

Part 1:

What is the purpose of the study?

Following on from your participation in the CATS I and CATS II studies, our research team and colleagues at the Cardiff University Brain Imaging Centre (CUBRIC) are hoping to undertake further studies to investigate how thyroid hormone levels during pregnancy might affect child brain development and function. In CATS II, you provided us with permission to collect a DNA sample from your child which we have used to study naturally occurring variation (or 'genotype') in a gene called deiodinase 2 (DIO2) which regulates thyroid function. We are interested in exploring any link between your thyroid function during pregnancy, your child's genotype and brain development. We are unsure of what the results will be as no one has studied this previously.

In this study, we plan to use advanced magnetic resonance imaging (MRI) scanning techniques and IQ tests to study brain structure and function in children.

Why have I and my child been chosen?

This study represents a follow-up study from the earlier CATS I and CATS II studies. It is only those involved in the previous studies that will be able to help us with our ongoing investigations. We are hoping to recruit 80 mother and child pairs to take part in this study.

Do I and my child have to take part?

No. Taking part is entirely voluntary. It is up to you and your child to decide whether or not you want to take part. We would ask you to read this document in detail before reaching a decision. You will also have the opportunity to ask questions, or clarify anything that isn't clear before making a decision.

If you decide that you do want to participate, we will ask you to sign a consent form. We will also ask your child to confirm that they are also happy to be involved, by asking them to sign an assent form. If your child does not agree to take part in the study, neither you nor your child will be included. Even after signing these forms, you are free to withdraw from the study at any time, and do not have to provide a reason or explanation for withdrawing. If requested, any unused samples will be disposed of according to locally approved procedures. Any samples used or results generated prior to the withdrawal of consent will continue to be utilised in this study.

Is there any reason why my child might not be able to take part?

Your child will not be able to take part if they have any fixed metal in or on them, for example a fixed dental brace or a pacemaker. The reason for this is the scanners use a strong magnetic field, and putting magnetic metal inside the scanner is dangerous. We will undertake a thorough safety questionnaire before proceeding with any scanning, to ensure that any potential dangers are identified and eliminated.

Where will the study take place?

The study will take place at the new Cardiff University Brain Research Imaging Centre (CUBRIC). This is a modern brain scanning research facility where multiple research projects are currently underway. The CUBRIC team are experienced in undertaking MRI studies in children. They use a number of approaches to reduce anxiety and minimise boredom, including playing videos during scanning, and using a 'mock scanner' beforehand so that children can familiarise themselves with the look and feel of the real MRI machine.

What is involved in this study if we decide to take part?

You and your child will be invited to attend CUBRIC for a 90minute session. CATS III; Mother information sheet. Version 3 Oct 2018

After you have agreed to take part in the study, we will be in contact by email or telephone with you to answer any questions you may have, check that there is no reason that your child cannot have the scans, arrange a convenient appointment time, and to check that you are happy to proceed. As well as the MRI scan for your childs, we will ask you to fill out a few forms whilst waiting. One of the forms your child is required to complete is about their current stage of puberty. It is important that we collect this information as this is a factor which influences brain development and may affect how we interpret the MRI scan results. We kindly request that you discuss this with your child before your appointment and explain that this is an important part of the data we will be collecting.

Your visit at CUBRIC will be similar to this:

1. With a research assistant, go over the participant information sheets, and discuss the project and any issues or concerns that you may have..
2. If you are happy to proceed, we will ask you to sign the consent form and your child to sign an assent form and fill out a puberty self-rating scale form.
3. Your child will complete a thorough brain scanning safety questionnaire with a research assistant.
4. You and your child will be taken to the mock MRI scanner for your child to acclimatise to the look and feel of the machine. When they are completely happy, a research assistant will take them to the real MRI machine to begin scanning. This will take approximately one hour, and your child can watch a film during this time if they wish.
5. While your child is undergoing their MRI scan, you will be asked to complete 3 questionnaires on your child's behaviour, as you did in the CATS II study.
6. At the end of the appointment, you and your child will have the opportunity to discuss any further questions you may have, and there will be a debrief sheet for your completion.

As in previous CATS studies, we can provide feedback for you and your child on the results of the IQ tests. You can of course be present during the test, but some children do not perform as well if their parents are there because they become distracted.

If your child finds being in the mock or real MRI scanner unpleasant, they can let us know straight away and we will stop the scanning immediately. More details regarding the scanning are outlined in part 2.

If you wish, we will be able to reimburse any travelling expenses /car parking fees incurred while attending for the study visits. And can offer a £15 Amazon voucher for attending.

What are the possible benefits of taking part?

There is no directly intended benefit to you or your child from taking part in this study. The scans are not intended to provide a medical diagnosis. They will not impact on medical treatment. The person conducting your scan will not be able to comment on the results of your scans.

The study aims to help us understand how exposure to thyroid hormones during pregnancy and your child's genetic make-up affects the development of the brain as well as intelligence.

What are the possible disadvantages and risks of taking part?

MRI scanning is a well-established form of imaging. No serious side-effects of being in an MRI scanner have been reported. Although the possibility of long-term effects cannot be

completely ruled out, many years of experience in this field would suggest MRI scans to be safe.
A few people have reported minor side-effects such as dizziness, mild nausea, metallic taste, or a sensation of seeing flashing lights. These side-effects, if experienced, go away as soon as the individual leaves the MRI scanner. If your child experiences any form of side-effect, they will be able to let us know immediately.
Although the modern scanners are much more compact than older models, some people find being inside an MRI scanner claustrophobic (meaning they become scared of being in a small or tight space). If your child doesn't like the experience of being in the mock scanner, or the real one for any reason, we will stop the scan straight away and take him/her out of the scanner.
Our tests may unexpectedly discover findings that have implications for your child. This may include things like abnormal changes on the scans (we call these incidental findings). If there are incidental scan findings, they will be looked at by a neuroradiologist (an expert in interpreting this type of scan) and we will contact you and your child's GP if necessary. There is additional information about this procedure in Part 2.
It is important to understand that these scans do not provide information that diagnose medical conditions. If you have health concerns about your child, you should contact a medical practitioner in the usual way.
Are the results confidential?
All information collected about you and your child during the course of this study will be kept strictly confidential. We may share the data we collect with other researchers, but any information that leaves the CUBRIC centre will have personal details (name, address etc) removed, so that the data is anonymous. In any report we publish, no information will be included that makes it possible for other people to know you or your child's identity in any way.
What will happen to the study results?
Where appropriate, the results of this study will be presented at medical and scientific conferences and published in journals. You and your child will not be identifiable from any report or publication. The results of this study will also help us to design and plan further research projects. CATS III; Mother information sheet. Version 3 Oct 2018

Part 2

If you have read part 1 of the information sheet and are interested in participating, please read the additional information in this section, which we hope will help you reach a decision.

What is an MRI scan?

MRI stands for magnetic resonance imaging. This is a technique that has been used for many decades, to image the body and the brain. It uses strong magnetic fields and low energy radio waves to take pictures of inside the human body in non-invasive ways. MRI does not involve radiation. There is no need for your child to have any drugs or medications. The study will not involve any form of injections.

An MRI scanner looks like this:

What does my child need to do before the scanning session?

There are no restrictions on lifestyle or diet before taking part in this study. We will not be asking your child to take or change any medical treatments. The scan can be quite long so we will ask your child if they require the toilet before going in to the scanner. Because MRI involves a very strong magnet, you and your child will be asked a series of safety questions. This is to make sure there is nothing in or on your child's body that might be affected by the scanner e.g. metal braces, jewellery, pacemakers.

We would suggest that your child comes in comfortable, loose clothing. It can be a little cool inside the scanning room, so warm clothing is advisable. If your child wishes to get changed before the scan, a private changing room will be available.

What will happen to my child during the MRI scan?

When you arrive for you appointment at CUBRIC, a research assistant will talk through what will happen with the MRI scans and there will be plenty of opportunity to ask questions. After this, you and your child will go the 'mock scanner' to see what it feels like to lie inside a scanner. Your child can stay in the mock scanner as long as he/she likes so that they will feel confident to go in the real one. In the mock scanner, which looks and feels identical to the real MRI scanner, your child can practice lying very still. We also suggest that your child practices this at home before the visit. CATS III; Mother information sheet. Version 3 Oct 2018

Once all the safety issues have been dealt with, if your child is happy to proceed, we will show them the real scanner. We will tell them how long each set of scans are likely to take (around 1 hour). We will then ask them to lie inside the scanner. The scanner consists of a large magnet with a tunnel, and your child will lie inside the tunnel. A radio-frequency coil will be placed around your child's head. These coils are used to transmit and receive radio waves (much like the waves used in radio or televisions).The MRI scanner does not use any form of radiation or x-rays.
The scanner can be noisy. Your child will be provided with ear plugs and/or ear defenders if they would like them.
We will ask your child to keep their head and body as still as possible whilst scanning is in progress.
The researchers will be present to ensure your child is comfortable, and will monitor your child carefully throughout the scanning process. They will be able to talk to your child via a microphone. Your child will be given a button he/she can press during the scan if they want to stop. As the scans last for an hour each, your child will be able to watch a film inside the MRI machine if they wish.
Are there any side-effects from the MRI scans?
MRI scanning has been used for many decades. It is widely considered to be a safe investigation, and most people do not experience side-effects.
A very small number of people have reported minor side-effects such as dizziness, mild nausea, an odd taste in their mouth, or seeing flashing lights. These side-effects, if experienced, would typically go away as soon as your child leaves the MRI scanner. If your child experiences any side-effects at all, they will be encouraged to let the researchers know as soon as possible.
In very rare cases, some people have reported a 'tickling' sensation in their back, shoulders or arms whilst inside an MRI scanner. This is not in any way harmful. However, if your child experiences this and wants us to stop, we will do so immediately.
Sometimes people become aware of their head and body warming up slightly during the scan. This is not harmful. We keep the scanner room fairly cool to keep your child as comfortable as possible.
What happens if you find something unusual on the scan?
The researchers involved are not experts in medical imaging of the brain. As such, they will not be looking to diagnose medical conditions. Occasionally when we image healthy participants, the researchers may be concerned regarding a potential abnormality. In this case, we will ask a neuroradiologist, with expertise in this field, to examine the scans, and if appropriate we will contact you and your GP, who can arrange further investigations/review as necessary. However, in the vast majority of cases a neurological consultant will not look at the images.
It is important to understand that these scans do not provide information that help in diagnosing medical conditions. If you have health concerns, you should contact a medical practitioner.
What happens to my child's samples at the end of the study? CATS III; Mother information sheet. Version 3 Oct 2018

Your child's samples may be retained at the end of this study for use in future research within the UK and abroad. At this stage we do not know what the research will involve. On the consent form you will be given the option to exclude your child's samples from future research. Your child's samples will not be sold for profit and will not be used in animal research or the commercial sector.

All samples will be supplied anonymously to researchers. Only Dr Rees and the study team will be able to identify which samples your child donated. The recipients of your samples will not be able to identify your child from the samples.

You may withdraw your consent for the storage and future use of your child's samples at any point. If you do withdraw your consent your child's samples will not be used in any subsequent studies and will be destroyed according to locally approved practices. Any samples already distributed will continue to be used in that study and will be destroyed at the end of the study.

Who is organising and funding the research?

The research is being organised by Dr Aled Rees at the Neurosciences and Mental Health Research Institute in Cardiff University. Funding for the study is provided by the American Thyroid Association and Waterloo Foundation. The doctors and researchers conducting the study are not being paid for including and looking after patients in the study.

Who has reviewed the study?

Before the study begins, the details of what the study involves will be thoroughly reviewed by a Research Ethics Committee. They review the details of the study to ensure that the research is conducted safely, fairly and appropriately. This study has been reviewed by The Cardiff & Vale University Health Board Research & Development Office and Wales Research Ethics Committee-1.

What if there is a problem?

If you are harmed by taking part in this research study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action, but you may have to pay for it.

What will happen to the results of the study?

The results of our study will be prepared for publication in medical/scientific journals, and for presentation at scientific conferences. All participants will be able to obtain a copy of the results once they have been published.

When our results are published or presented, individual data will be anonymised, so that it is not possible for others to identify you or your child as having been involved in the research.

Will my GP be told that I and my child are taking part in the study?

With your consent we will write to your GP to inform them that you have taken part in this study.

Future Studies

It is likely that further research will be carried out related to the CATS study. If this happens, we may try to contact you at some point in the future, to see whether you would be interested in participating again.

Further questions CATS III; Mother information sheet. Version 3 Oct 2018

If you have any concerns or complaints about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions or you can contact Dr Aled Rees on 02920 745002 or by email (ReesDA@cardiff.ac.uk). CATS III; Mother information sheet. Version 3 Oct 2018 CATS III; Mother information sheet. Version 3 Oct 2018

CATS III

Introduction

You have been contacted to see whether you might be interested in taking part in a research study. This document tells you a little bit about this study, and will hopefully help you decide whether you want to join in. Your mum might be able to help you reach a decision.

What is research?

Research is a way of trying to find out the answers to important questions.

What questions are you trying to answer?

Everybody has a gland in their neck called the thyroid gland. This gland makes a chemical called thyroid hormone. Thyroid hormone is very important because it helps many parts of the body to work properly. For example, thyroid hormones make sure the cells in your body work at the right speed; if they worked too quickly, you would feel hot and sweaty a lot of the time, if they worked too slowly, you would feel tired and cold. We hope that this study will help us understand how thyroid hormone helps the brain to develop during childhood.

Why have I been invited to take part?

You have been specially chosen because you and your mother have been involved in this research before. Your mother had some tests whilst she was pregnant with you, and when you were around age 9 you helped us by having some tests, and doing some complicated puzzles. Now that you are a little older, we would like you to help us find out a little bit more.

There is nothing wrong with you or your mum. Everybody has a thyroid gland. You have been specially chosen because you have helped us in the past.

Do I have to take part?

No. It is entirely your choice whether you would like to take part. Even if you do decide to get involved, you can change your mind at any time.

Did anyone check that this study is ok to do?

Before we undertake any research, a group of people called the Research Ethics Committee check the study carefully, to make sure it is safe and fair. Your project has been checked by the South East Wales Research Ethics Committee.

What will I be asked to do if I decide to take part?

If you want to take part, we will ask you to come to visit us with your mum. At your appointment, we will do some puzzles with you, scan your head, ask you to fill out some questionnaires, and with your permission, we will take a blood and urine sample from you.

Where do I have to go?

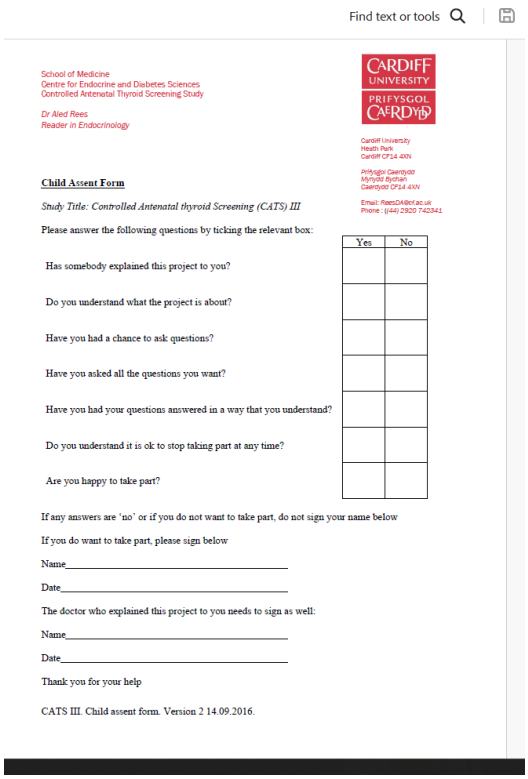
If you are happy to take part, we will ask you and your mum to come to visit us at the new CUBRIC centre, which is a research centre where the scanner is kept. When you come to visit us, we will talk to you about the research in a lot more detail, and answer any questions you might have. If you are happy to join in, we will ask you and your mum to sign a form to say that you want to take part. You can still change your mind at any time.

What do the puzzles involve? CATS III; Mother information sheet. Version 3 Oct 2018

The puzzles are similar to the ones you completed when you were a little bit younger. They will take around one hour and will check your learning and concentration.
What does the scan involve?
There will be two separate scans, both lasting for about 1 hour each. Before you have the scan, we will show you a ‘pretend’ version of the scanner, so that you can see what it looks like, and see what it feels like to lie in it.
The scanner looks a little bit like this:
We will ask you to lie inside the scanner, trying to keep your head still for about 1 hour in total. As you may get a little bored during scanning, we can play you a film to watch (you can choose from a selection we have at the research centre).
We will be able to speak to you throughout the scan. It can be a little noisy in the scanner, so we will offer you ear plugs that you can use if you want. We will also give you a button that you can press if you want to stop at any time. We will be able to see you, to make sure you are comfortable all of the time.
Is the scan going to hurt me?
No, the scan will not hurt you. This type of scan is done all of the time, and no serious side-effects have been reported.
A few people have reported that they have felt a little bit dizzy, or a little bit sick whilst they are having the scan. If you feel funny in any way during the scan, let us know and we can stop straight away. The few people who have had these side-effects, have felt completely normal as soon as they come out of the scanner.
There are no known long-lasting side-effects associated with these scans.
Will taking part help me?
We don’t expect that the results of this study will help you personally. However, the information we get from this research might help us treat pregnant mums with problems with their thyroid in the future, and help their children when they are growing up.
What happens when the research stops?
This study does not involve taking tablets so nothing will change for you. CATS III; Mother information sheet. Version 3 Oct 2018

What if something goes wrong during the project?
This is very unlikely. The scan we will be doing is very safe. We are not going to give you any medicines or treatments.
Will my medical details be kept private if I take part?
Yes, absolutely. Only the doctors involved in the study will know about you taking part. Any information we collect from you will not have your name or address on it. The blood and urine samples (if you choose to provide them), will be stored at Cardiff University and only members of the research team will have access to them. With the blood samples, we will check if your thyroid is working OK, and with the urine we will measure iodine levels, which can affect how your thyroid works.
What if I change my mind about taking part?
You can change your mind at any time, if you don't want to take part anymore. Just let your parents, doctor or nurse know. Nobody will be cross with you if you want to stop.
Who can I speak to if I want to ask more questions?
You can contact Aled Rees if you have any questions on 02920 745002 or if you prefer, you can email him at: ReesDA@cardiff.ac.uk or Anna Scholz, the research assistant on ScholzA6@cardiff.ac.uk.

Appendix



Find text or tools

School of Medicine
Centre for Endocrine and Diabetes Sciences
Controlled Antenatal Thyroid Screening Study

Dr Aled Rees
Reader in Endocrinology

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Email: ReesDA@cf.ac.uk
Phone : (44) 2920 742341

Child Assent Form

Study Title: Controlled Antenatal thyroid Screening (CATS) III

Please answer the following questions by ticking the relevant box:

	Yes	No
Has somebody explained this project to you?		
Do you understand what the project is about?		
Have you had a chance to ask questions?		
Have you asked all the questions you want?		
Have you had your questions answered in a way that you understand?		
Do you understand it is ok to stop taking part at any time?		
Are you happy to take part?		

If any answers are 'no' or if you do not want to take part, do not sign your name below

If you do want to take part, please sign below

Name_____

Date_____

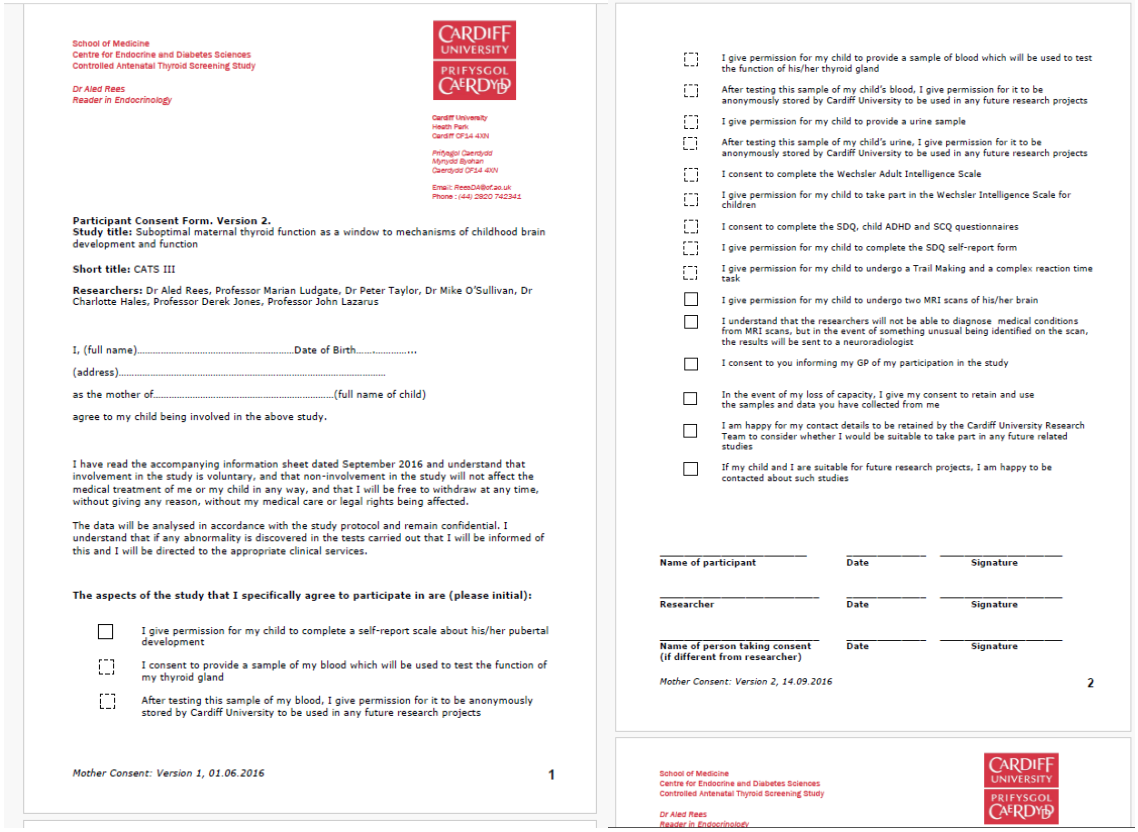
The doctor who explained this project to you needs to sign as well:

Name_____

Date_____

Thank you for your help

CATS III. Child assent form. Version 2 14.09.2016.



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Phone : (44) 2920 742341

Participant Consent Form. Version 2.
Study title: Suboptimal maternal thyroid function as a window to mechanisms of childhood brain development and function

Short title: CATS III

Researchers: Dr Aled Rees, Professor Marian Ludgate, Dr Peter Taylor, Dr Mike O'Sullivan, Dr Charlotte Hales, Professor Derek Jones, Professor John Lazarus

I, (full name).....Date of Birth.....,,,

(address).....

as the mother of.....(full name of child)

agree to my child being involved in the above study.

I have read the accompanying information sheet dated September 2016 and understand that involvement in the study is voluntary, and that non-involvement in the study will not affect the medical treatment of me or my child in any way, and that I will be free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

The data will be analysed in accordance with the study protocol and remain confidential. I understand that if any abnormality is discovered in the tests carried out that I will be informed of this and I will be directed to the appropriate clinical services.

The aspects of the study that I specifically agree to participate in are (please initial):

☐ I give permission for my child to complete a self-report scale about his/her pubertal development

☐ I consent to provide a sample of my blood which will be used to test the function of my thyroid gland

☐ After testing this sample of my blood, I give permission for it to be anonymously stored by Cardiff University to be used in any future research projects

Mother Consent: Version 1, 01.06.2016 1

☐ I give permission for my child to provide a sample of blood which will be used to test the function of his/her thyroid gland

☐ After testing this sample of my child's blood, I give permission for it to be anonymously stored by Cardiff University to be used in any future research projects

☐ I give permission for my child to provide a urine sample

☐ After testing this sample of my child's urine, I give permission for it to be anonymously stored by Cardiff University to be used in any future research projects

☐ I consent to complete the Wechsler Adult Intelligence Scale

☐ I give permission for my child to take part in the Wechsler Intelligence Scale for children

☐ I consent to complete the SDQ, child ADHD and SCQ questionnaires

☐ I give permission for my child to complete the SDQ self-report form

☐ I give permission for my child to undergo a Trail Making and a complex reaction time task

☐ I give permission for my child to undergo two MRI scans of his/her brain

☐ I understand that the researchers will not be able to diagnose medical conditions from MRI scans. but in the event of something unusual being identified on the scan, the results will be sent to a neuroradiologist

☐ I consent to you informing my GP of my participation in the study

☐ In the event of my loss of capacity, I give my consent to retain and use the samples and data you have collected from me

☐ I am happy for my contact details to be retained by the Cardiff University Research Team to consider whether I would be suitable to take part in any future related studies

☐ If my child and I are suitable for future research projects, I am happy to be contacted about such studies

Name of participant	**Date**	**Signature**
Researcher	**Date**	**Signature**
Name of person taking consent (if different from researcher)	**Date**	**Signature**

Mother Consent: Version 2, 14.09.2016 2

School of Medicine
Centre for Endocrine and Diabetes Sciences
Controlled Antenatal Thyroid Screening Study

Dr Aled Rees
Reader in Endocrinology

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