Common variation in thyroid hormone status; effects on key health outcomes

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This thesis is submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (PhD)

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Submitted May 201

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Summary

Chronic pathological variation in thyroid function has major adverse outcomes on adult health, pregnancy and childhood development. However, it is less clear whether more minor variation, including variation across and just outside the reference-range has sufficient impact to justify intervention in selected individuals.

Aims

To investigate the relationship between modest variation in thyroid function on health outcomes, and how our treatment of hypothyroidism may relate to this, including screening for and treating low thyroid function in pregnancy.

Scope

I investigated the relationship between variation in thyroid function within the normal reference-range on health outcomes and identified that lower thyroid function was associated with adverse cardiovascular and metabolic outcomes and higher thyroid function was associated with adverse bone outcomes. I also identified in the ALSPAC cohort that TSH, FT3 and FT4 were all associated with body composition. However, FT3 was surprisingly positively associated with fat mass and genetic analyses indicated that higher fat mass was driving the higher FT3 levels.

I then investigated current UK management of hypothyroidism utilising a large primary care database. Here I demonstrated a falling TSH threshold at levothyroxine initiation and a high risk of over-treatment. Individuals with depression or tiredness were more likely to be over-replaced. Women with suboptimal replacement during pregnancy had a higher risk of foetal loss. I then utilised data from the CATS trial and data linkage via SAIL to demonstrate that screening for and treating low thyroid function during pregnancy reduces foetal loss.

Conclusion

Common variation in thyroid status appears to be a modifiable risk factor for adverse health outcomes. Targeted treatment in patient sub-groups may provide substantial benefit. Furthermore, FT3 appears to be more fluid and influenced by external factors. Further research into novel methods of assessing tissue thyroid hormone levels may provide clarity to the treatment of borderline thyroid function.

Acknowledgements

I must thank Professor Colin Dayan whose encouragement, enthusiasm and wisdom, introduced me to research and guided me through my PhD Studies. I also particularly thank Dr. Timpson for similar wisdom and enthusiasm in mentoring and guiding me through epidemiology and genetic epidemiology. My thanks also to Dr. Onyebuchi Okosieme, Professor John Lazarus, Dr. Aled Rees and Professor Marion Ludgate for their ongoing support and invaluable advice over my PhD. Lastly, but by no means least I must thank my family for their support and inspiration in undertaking this work.

Papers related to this thesis published to date

Global epidemiology of hyperthyroidism and hypothyroidism
Peter N Taylor*, Diana Albrecht*, Anna Scholz*, Gala Gutierrez-Buey, John H Lazarus, Colin M Dayan, Onyebuchi E Okosieme
Nature Reviews Endocrinology 2018

Debate: Identifying and Treating Subclinical Thyroid Dysfunction in Pregnancy: Emerging controversies
Ines Velasco, Peter N Taylor
European Journal of Endocrinology 2017

Levothyroxine in Obstetrics
Ines Velasco, Peter N Taylor
Ann Med. 2017 Oct 3:1-29

Maturation in serum thyroid function parameters over childhood and puberty: results of a longitudinal study
Peter N Taylor, Adrian Sayers, Onyebuchi Okosieme, Gautam Das, Mohd S Draman, Arshiya Tabasum, Hussam Abusahmin, Mohammad Rahman, Kirsty Stevenson Alix Groom, Kate Northstone, Wolf Woltersdorf, Andrew Taylor, Susan Ring, John H Lazarus, John W Gregory, Aled Rees, Nicholas Timpson, Colin M Dayan
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Peter N Taylor, Rebecca Richmond, Neil Davies, Adrian Sayers, Kirsty Stevenson, Wolfram Woltersdorf, Andrew Taylor, Alix Groom, Kate Northstone, Susan Ring, Onyebuchi Okosieme, Aled Rees, Dorothea Nitsch, Graham R Williams, George Davey-Smith, John W Gregory, Nicholas J Timpson, Jonathan H Tobias, Colin M Dayan
JCEM 2016

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Peter N Taylor, Ahmed Iqbal, Caroline Minassian, Adrian Sayers, Mohd Draman, Rosemary Greenwood, William Hamilton, Onyebuchi Okosieme, Vijay Panicker, Sara Thomas, Colin Dayan
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Peter N Taylor, Salman Razvi, Simon Pearce, Colin M Dayan
JCEM 2013

Other key papers published during PhD

Combined immunosuppression & radiotherapy in thyroid eye disease (CIRTED): a multi-centre, factorial randomised controlled trial
Rathie Rajendram*, Peter N Taylor*, Victoria J Wilson, Nicola Harris, Olivia C Morris, Marjorie Tomlinson, Sue Yarrow, Helen Garrott, Helen Herbert, Andrew D Dick, Anne Cook, Rao Gattamaneni, Rajni Jain, Jane Olver, Steven Hurel, Fion Bremner, Suzannah R Drummond, Ewan Kemp, Diana M Ritchie, Nichola Rumsey, Daniel Morris, Carol Lane, Nachi Palaniappan, Chunhei Li, Julie Pell, Robert Hills, Daniel Ezra, Mike J Potts, Sue Jackson, Geoff E Rose, Nicholas Plowman, Catey Bunce, Jimmy M Uddin, Richard WJ Lee, Colin M Dayan
Lancet Diabetes and Endocrinology 2018

Iodine supplementation in pregnancy – is it time ?
Peter N Taylor, Bijay Vaidya
Clinical Endocrinology 2016

Whole genome sequence based analysis of thyroid function
Peter N Taylor*, Eleonora Porcu*, Shelby Chew*, Purdey J. Campbell*, Michela Traglia[6], Suzanne J. Brown, Benjamin H. Mullin, Hashem A. Shihab, Josine Min, Klaudia Walter, Yasin Memari, Jie Huang, Michael R. Barnes, John P. Beilby, Pimphen Charoen, Petr Danecek, Frank Dudbridge, Vincenzo Forgetta, Celia Greenwood, Elin Grundberg, Andrew D. Johnson, Jennie Hui, Ee M. Lim, Shane McCarthy, Dawn Muddyman, Vijay Panicker, John R.B. Perry, Jordana T. Bell, Wei Yuan, Caroline Relton, Tom Gaunt, David Schlessinger, Goncalo Abecasis, Francesco Cucca, Gabriela L. Surdulescu, Wolfram Woltersdorf, Eleftheria Zeggini, Hou-Feng Zheng, Daniela Toniolo, Colin M. Dayan, Silvia Naitza, John P. Walsh, Tim D. Spector, George Davey Smith, Richard Durbin, J. Brent Richards, Serena Sanna, Nicole Soranzo, Nicholas J. Timpson*, Scott G. Wilson* and the UK10K Consortium. *Authors contributed equally
Nature communications 2015

Genetic abnormalities in thyroid hormone deiodinases
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Peter N Taylor, Onyebuchi E Okosieme, Rhian Murphy, Charlotte Hales, Elisabetta Chiusano, Aldo Maina, Mohamed Joomun, Jonathan P Bestwick, Peter Smyth, Ruth Paradice, Sue Channon, Lewis E Braverman, Colin M Dayan, John H Lazarus, Elizabeth N Pearce
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European Journal of Endocrinology 2013

Side effects of anti-thyroid drugs and their impact on the choice of treatment for thyrotoxicosis in pregnancy.
Peter N Taylor Bijay Vaidya
European Thyroid Journal 2012

Key oral presentations relating to this thesis

Apr 2018 Controlled Antenatal Thyroid Screening Study: Obstetric Outcomes
The Association of Physicians Of Great Britain
(awarded best oral presentation)

Nov 2017 How to define Normal TSH in Pregnancy
European Society of Endocrinology Course on Endocrine Diseases in Pregnancy, Krakow (**invited presentation**)

Sept 2017 Thyroid in Pregnancy
Latvian Endocrine Society (**invited presentation)**

Nov 2016 Controlled Antenatal Thyroid Study; Obstetric Outcomes
British Endocrine Society

Sept 2016 Controlled Antenatal Thyroid Study; update on projects
American Thyroid Association

Sept 2016 Clinical Educational course – Pathways of Progression to Overt Hypothyroidism from Subclinical Hypothyroidism
European Thyroid Association (**invited presentation)**

May 2016 Controlled Antenatal Thyroid Study; Obstetric Outcomes
British Thyroid Association (**highest scoring abstract**)

May 2016 Thyroid and Pregnancy Update
British Thyroid Association (**invited presentation**)

Dec 2014 Paradoxical relationship between body mass index and thyroid hormone levels in children; a study using Mendelian Randomization
British Thyroid Association
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Sept 2014 Increased fat mass appears to result in higher free T3 levels in children
European Thyroid Association

Nov 2013 Thyroid Function and Body Composition in Children: cause or effect? A study using Mendelian Randomization
British Thyroid Association (**invited presentation**)

Nov 2013 Patients with serum TSH above the normal reference range should be given thyroid hormone replacement.
British Thyroid Association (**invited debate presentation**)

Nov 2013 Thyroid function monitoring and TSH levels in pregnant individuals on levothyroxine in the UK
British Thyroid Association

Oct 2013 Falling threshold for treatment of borderline elevated TSH levels - balancing benefits and risks: evidence from a large community based study
Welsh Endocrine and Diabetes Society

Sept 2013 Trends in thyroid hormone prescribing in pregnancy and clinical outcomes by TSH level.
European Thyroid Association

Abbreviations used in this thesis

ALSPAC	Avon Longitudinal Study of Parents and Children
BMI	Body Mass Index
CATS	Controlled Antenatal Thyroid Study
CPRD	Clinical Practice Research Datalink
DIO1	Deiodinase 1 enzyme
DIO2	Deiodinase 2 enzyme
DXA	Dual-energy X-ray absorptiometry
FMI	Fat mass index
FT3	Free Tri-iodothyronine
FT4	Free Thyroxine
GH	Growth Hormone
HPT	Hypothalamus-Pituitary-Thyroid
IH	Isolated Hypothyroxinemia
IQR	Inter-quartile Range
IV	Instrumental Variable
OR	Odds Ratio
MI	Myocardial Infarction
N	Number
P	P value
RR	Relative Risk
SAIL	Secure Anonymised Information Linkage
SCH	Subclinical Hypothyrodisim
STANDARDISED	Standardised
TSH	Thyroid Stimulating Hormone
TPO	Thyroid peroxidase
UIC	Urinary Iodine Concentration
YRS	Years
95% CI	95% Confidence Interval

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Chapter 1 – Introduction

Thyroid status is critical to childhood development and adult health. Whilst overt thyroid disease has substantial negative health consequences, it is unclear whether more modest variation in thyroid status has sufficient impact on health to warrant intervention. This is an important issue to address as thyroid disease is easily treatable and borderline thyroid dysfunction is common in the general population.

In particular, thyroid hormone has substantial effects on cardiovascular risk factors, metabolism, bone maintenance, mental health, as well as pregnancy outcomes and childhood development [1-3]. Effects are likely to be causal as outcomes are influenced by thyroid hormone replacement or anti-thyroid medication. Furthermore, pregnancy is a period of key vulnerability to sub-optimal thyroid function although data on early intervention are limited [4].

Taken together there is a pressing need to investigate the impact of common variation in thyroid status including variation across the normal population. Given that hypothyroidism is particularly common, and widely tested for, the impact or current UK practice will also be assessed. As the majority of studies to date have been performed in adults I will also explore the effect of variation in thyroid status on outcomes in children to assess the effect of variation in thyroid home during a key

time in development. In this thesis, I will therefore assess the following key areas:

1) The effect of common variation in thyroid hormone status within the population reference range on key health outcomes using existing published data from epidemiological cohorts.
2) The relationship between thyroid function and body composition in children using data from the Avon Longitudinal Study of Parents and Children (ALSPAC) to assess the impact of common variation in thyroid function in children.
3) UK practice regarding thyroid hormone replacement in primary hypothyroidism, with a focus on its management and consequences during pregnancy, using primary care registry data.
4) The benefits of screening for and correcting low thyroid function in pregnancy using data linkage and the Controlled Antenatal Thyroid Study (CATS) trial.

1.1 THYROID HORMONE EFFECTS

Thyroid hormones have fundamental but diverse physiological roles across vertebrate species, ranging from photoperiodic regulation of seasonal breeding in birds to induction of metamorphosis in amphibians [5]. In humans, they act on almost all nucleated cells and are essential for normal growth and differentiation, as well as regulation of energy metabolism and correct physiological function. The consequences of pathological thyroid dysfunction (hyper/hypothyroidism) can be profound [1, 2]. Extreme hypothyroidism, myxoedema coma is a rare endocrine emergency with a high mortality rate of 25-70% [6] similarly poor outcomes are observed with extreme hyperthyroidism -thyroid storm [1]. The consequences of hypo and hyperthyroidism are summarized in **Table 1**.

Table 1 Signs and Symptoms of Hyper- and Hypothyroidism

Hyperthyroidism	Hypothyroidism
Hyperactivity	Slowing of mental functions
Emotional lability	Emotional lability
Insomnia	Inability to concentrate, poor memory
Fatigue	Fatigue, lethargy
Anxiety	Depressive symptoms
Weight loss despite increased appetite	Weight gain despite decreased appetite
Heat intolerance	Cold intolerance
Palpitations	Deranged lipid profile
Atrial fibrillation	Heart failure
Excess sweating	Decreased sweating
Dry Skin	Dry skin, Hair loss
Diarrhea	Constipation
Muscle weakness	Muscle weakness
Osteoporosis	Joint pain
Lighter menses	Heavier Menses
Impaired fertility	Impaired fertility

1.2 EPIDEMIOLOGY OF THYROID DISEASE

Hypothyroidism and hyperthyroidism are common conditions that affect all populations worldwide. Iodine nutrition is a key determinant of thyroid disease risk [7]; however, other factors such as ageing [1], smoking status [8], genetic susceptibility [9], ethnicity [10], endocrine disruptors [11] also influence thyroid disease epidemiology. These factors are summarized in **Table 2**.

Table 2 Risk factors for developing Hypothyroidism and Hyperthyroidism

Risk factor	Hypothyroidism	Hyperthyroidism	Comment
Female sex	+	+	Sex hormones and the skewed inactivation of the X chromosome are suspected to be triggers for hypothyroidism and hyperthyroidism [12].
Iodine deficiency	+	+	Severe iodine deficiency can cause hypothyroidism and hyperthyroidism [13].
Iodine excess	+	+	Excess iodine status can trigger hyperthyroidism typically in elderly individuals with longstanding thyroid nodules and hyperthyroidism [13].
Transition from iodine deficiency to sufficiency	+	+	Transition from iodine deficiency to sufficiency was associated with an increase in thyroperoxidase antibodies, one study reported an increase from 14.3% to 23.8% [14].
Other autoimmune conditions	+	+	One study reported that another auto-immune disease was present in almost 10% of patients with Graves' disease and in 15% of patients with Hashimoto's thyroiditis, with rheumatoid arthritis being the most common [15].

Risk Factor	Hypothyroidism	Hyperthyroidism	Comment
Genetic risk factors	+	+	Both Graves' disease and Hashimoto thyroiditis have genetic predispositions. Genome wide association data have identified regions associated with thyroperoxidase antibody positivity [16] and thyroid disease [16].
Smoking	–	+	Current smoking increases the odds of Graves' hyperthyroidism almost 2-fold and increases the risk of Graves' ophthalmopathy almost 8-fold [17]. Smokers also have a slower response during anti-thyroid drug treatment [18]. Smoking might protect against hypothyroidism [19, 20].
Alcohol	–	n/a	Moderate alcohol intake might be associated with a reduced risk of hypothyroidism [21].
Selenium deficiency	+	+	One study reported that patients with newly diagnosed Graves' disease and hypothyroidism had lower selenium levels than the normal population [22].
Drugs	+	+	Examples of drugs that can cause hyperthyroidism and hypothyroidism include Amiodarone [23], Lithium [24] and interferon γ.
Syndromic conditions	+	n/a	Almost 25% of patients in a large registry of patients with Down syndrome had thyroid disease, the most common being primary hypothyroidism [25]. The prevalence of hypothyroidism in Turner syndrome is approximately 13% [26].

–, reduced risk; + increased risk, n/a unclear if a risk factor

Iodine deficiency and auto-immune disease (known as Hashimoto thyroiditis) account for the vast majority of cases of primary hypothyroidism [2]. A third of the world's population live in iodine deficient areas and the devastating consequences of severe iodine deficiency on the neurological development of fetuses and children are well recognized [7].

In iodine sufficient countries, the prevalence of hypothyroidism ranges from 1–2% [27, 28], rising to 7% in individuals aged between 85–89 years [29]. In the absence of age-specific reference ranges for TSH, an ageing population is likely to result in a higher prevalence of hypothyroidism. Hypothyroidism is approximately 10 times more prevalent in women than men [27]. In the developed world, the prevalence of undiagnosed overt hypothyroidism is likely falling due to widespread thyroid function testing [30]. Data from Norway showed that the prevalence of untreated overt hypothyroidism was low at 0.1%, reflecting a fall of 84% from the 1990s [30].

The prevalence of overt hyperthyroidism ranges from 0.2% to 1.3% in iodine sufficient parts of the world [12, 31]. In 1977, the UK Whickham study reported that the incidence of hyperthyroidism was estimated at between 100–200 cases per 100,000 per year with a prevalence of 2.7% in women and 0.23% in men, taking into account both established and possible cases[32]. These figures were considerably higher than earlier retrospective data from the USA which reported an incidence of 30 cases

per 100,000 a year for Graves' disease in the period 1935–1967 [33]. A 20-year follow up of the Whickham cohort showed an ongoing incidence of 80 cases per 100,000 women per year [31, 34]. In the 2002, United States National Health and Nutrition Examination Survey (NHANES III), overt hyperthyroidism was detected in 0.5% of the population while 0.7% of the population had subclinical hyperthyroidism [31] with an overall prevalence of 1.3%. Studies from several other countries including Sweden [35, 36], Denmark [37], Norway [38] and Japan [39] have all reported comparable incidence and prevalence rates. A meta-analysis of European studies estimated a mean prevalence rate of 0.75% and an incidence rate of 51 cases per 100,000 per year [12].

An overview of the prevalence of hypothyroidism based on epidemiological surveys, is summarized in **Table 3** and **Figure 1**. Most of the available data are from Europe and North America and data from Africa in particular is lacking [40]. These data show that hypothyroidism is common throughout the world, and is particularly common in the UK. This raises the possibility that the UK may also have a substantial number of individuals with thyroid function in the lower point of the population reference range.

The prevalence of overt hypothyroidism in the general population ranges between 0.2% and 5.3% in Europe [30, 41] and 0.3% and 3.7% in the USA [42] depending on the definition used and population studied (**Table 3**). Longitudinal studies from large UK cohorts report an incidence rate of spontaneous hypothyroidism of 3.5–5.0 per 1000 per year in women and 0.6 – 1.0 per 1000 per year in men [34, 43]. A survey conducted in Spain reported a prevalence of treated hypothyroidism, untreated subclinical hypothyroidism, and untreated clinical hypothyroidism of 4.2%, 4.6% and 0.3%, respectively [44]. A 2010 study from Australia reported the five-year incidence of hypothyroidism in individuals aged >55 years was 0.5% and 4.2%, respectively[45], while the prevalence of overt and subclinical hypothyroidism was estimated at 0.5% and 5.0%, respectively [46]. The longest follow-up study is from the UK Whickham cohort [32, 34], where the mean annual incidence of spontaneous hypothyroidism during a 20-year follow-up period was 35 cases per 10,000 surviving women and 6 per 10,000 surviving men [34]. Higher TSH levels and the presence of thyroid

antibodies were associated with an increased risk of developing hypothyroidism with a positive interactive effect [34].

Table 3 Prevalence of hypothyroidism in iodine sufficient and iodine deficient countries

Author, country, publication year	Study date	Sample size (N)	Age (yrs)	Female (%)	Iodine intake/UIC	Prevalence, %		
						M	F	Total
IODINE SUFFICIENT								
Tunbridge, UK, 1977[32]	1972-1974	2,779	>18	54	811 nmol/24h	0.1	1.4	1.8
Konno, Japan, 1993 [39]	1990-1991	4,110	Adult	29	n/a	0.7	3.1	n/a
Galofre, Spain, 1994 [47]	1990-1992	103,098	15-85	57	n/a	n/a	n/a	n/a
Vanderpump, UK, 1995[34]	1975-1994	1,877	38-93	56	102µg/g-cr	1.3	9.3	5.8
Bjoro, Norway, 2000[38]	1995-1997	94,009	>20	50	n/a	0.4	0.8	0.7
Canaris, USA, 2000[42]	1995	24,337	>18	56	n/a	n/a	n/a	0.4
Hollowell, USA, 2002[31]	1988-1994	13,344	>12		145 µg/l	n/a	n/a	0.3
Volzke, Germany, 2003[48]	1997-2001	3,941	20-79	48	12µ g/dL	n/a	n/a	0.7
Flynn, UK, 2004[43]	1993-1997	369,885	>0	n/a	n/a			3.0
O' Leary Australia 2006[49]	1981	2,115	16-89	50	n/a	0.4	0.7	0.54
Teng†, China, 2006 (total) [50]	1999	3761 (total)	≥18	69	n/a	n/a	n/a	n/a
Teng†, China, 2006 (excess) [50]	1999	1074	≥18	n/a	n/a	n/a	n/a	2.0
Teng†, China, 2006 (sufficient) [50]	1999	1584	≥18	n/a	n/a	n/a	n/a	0.9
Sichieri, Brazil, 2007 [51]	2004-2005	1200 (white)	≥35	100	n/a	n/a	1.6	n/a
Sichieri, Brazil, 2007 [51]	2004-2005	(Mixed)	n/a	n/a	n/a	n/a	1.3	n/a
Sichieri, Brazil, 2007 [51]	2004-2005	(Black)	n/a	n/a	n/a	n/a	0.6	n/a
Leese, UK, 2007[52]	1994-2001	388,750	>0	52	n/a	1.0	5.5	3.0
Kasagi, Japan, 2009[53]	2005-2006	1818	51.3+/-9.0	56	n/a	0.2	0.5	0.66
Lucas, Spain, 2010[54]	2002	1,124	18-74	56	150 µg/l	0	0.5	0.2

Author, country, publication year	Study date	Sample Size (N)	Age (yrs)	Female (%)	Iodine intake/UIC	Prevalence, % M	F	Total
Asvold, Norway, 2012[30]	1995-2008	15,106	>20	67	n/a	n/a	n/a	n/a
Marwaha, India, 2012[55]	2007-2010	4402	18-90	63	n/a	n/a	n/a	4.2
Delshad, Iran, 2012 [56]	1999-2005	1,999	>20	61	n/a	n/a	n/a	
Unnikrishnan, India, 2013*[57]	2011	5376	18-100	54	n/a	n/a	n/a	10.95
Sriphrapradang, Thailand, 2013[58]	2009	2545	≥14	n/a	n/a	n/a	n/a	0.74
IODINE DEFICIENT								
Laurberg, Denmark, 1999[59]	24 months	569,108	>0	51	60 µg/day	n/a	n/a	n/a
Aghini-Lombardi, Italy, 1999[60]	1995	992	>15	58	55 µg/l	0	0.3	0.2
Knudsen, 1999, Denmark[61]	1993-1994	2,613	41-71	49	70 µg/l	0.2	0.5	0.3
Knudsen, 2000, Denmark[37]	1997-1998	2,293	18-65	79	45 µg/l	n/a	n/a	0.2
Knudsen, 2000, Denmark[37]	1997-1998	2,067	18-65	79	61 µg/l	n/a	n/a	0.6
Hoogendoorn, 2006, Netherlands[62]	2002-2003	5,167	>18	54	n/a	0.2	0.6	0.4
Laurberg, 2006, Denmark[63]	1997-1998	310,124	18-65	50	68 µg/l	n/a	n/a	n/a
Laurberg, 2006, Denmark[63]	1997-1998	225,707	18-65	53	53 µg/l	n/a	n/a	n/a
Teng†, China, 2006 (deficient) [50]	1999	1103	≥18	n/a	n/a	n/a	n/a	0.3
Du, China, 2014 (mildly deficient) [64]	n/a	667	≥18	71	n/a	0.2	0.9	1.05

Data is for cases of overt hypothyroidism except where otherwise stated. Iodine status is based on reported status by authors; spaces are left blank where there is no data on prevalence or where the data is unclear from the report. Studies in specific population groups such as children, pregnant women, specificied co-morbid states, and unstable iodine nutrition are excluded. UIC, urinary iodine concentrations

†Same study population, studied at 5 and 11 year intervals post iodization. Data in follow-up available on excess replacement as in some individuals excess levels were recorded (median in this group, 651 microg per liter).*Data from 8 cities with a wide mix of iodine status from sufficient to deficient.

Figure 1 Global epidemiology of hypothyroidism

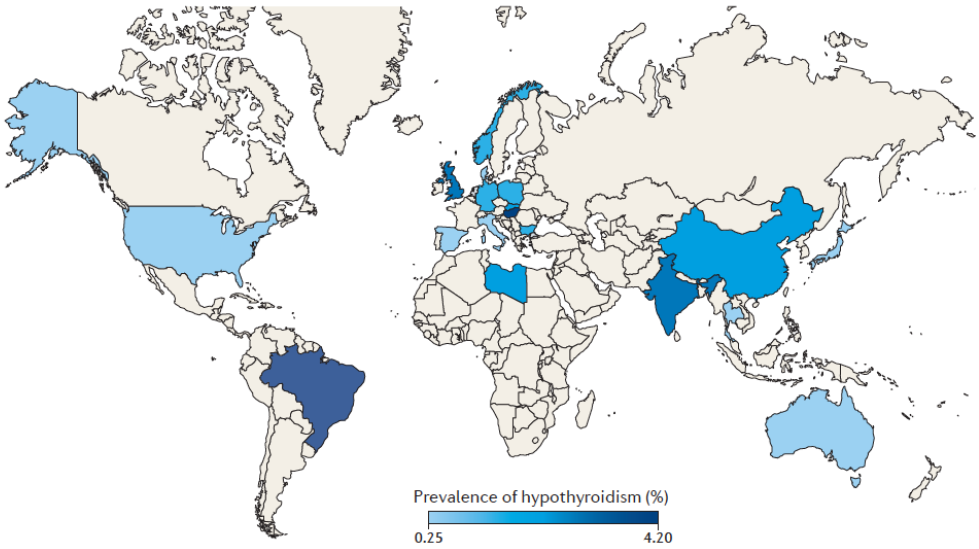


Figure created using Tableau software version 10.3. World map showing global prevalence of hypothyroidism based on epidemiological surveys. If multiple studies have been performed on the prevalence of hypothyroidism from one country, the median value was used. The deeper the shade of blue the higher the prevalence of hypothyroidism.

Hyperthyroidism is less common than hypothyroidism but also has an uneven distribution globally. Its prevalence and incidence of thyroid dysfunction is difficult to compare across countries due to differences in diagnostic thresholds, assay sensitivities, population selection, and fluxes in iodine nutrition and population dynamics although epidemiological surveys are still available (**Table 4, Figure 2**). As with hypothyroidism, the majority of studies are from Europe and North America. The prevalence of overt hyperthyroidism is roughly similar in Europe and the United States (0.7 versus 0.5%) [12, 31] although the prevalence of overt hyperthyroidism ranges from 0.2% to 1.3% in iodine sufficient parts of the world[12, 31] (**Table 4**). In 1977, the UK Whickham study reported that the incidence of hyperthyroidism was estimated at between 100–200 cases per 100,000 per year with a prevalence of 2.7% in women and 0.23%

in men, taking into account both established and possible cases [32]. These figures were considerably higher than earlier retrospective data from the USA which reported an incidence of 30 cases per 100,000 a year for Graves' disease in the period 1935–1967 [33]. A 20-year follow up of the Whickham cohort showed an ongoing incidence of 80 cases per 100,000 women per year [31, 34]. In the 2002, United States National Health and Nutrition Examination Survey (NHANES III), overt hyperthyroidism was detected in 0.5% of the population while 0.7% of the population had subclinical hyperthyroidism [31] with an overall prevalence of 1.3%. Studies from several other countries including Sweden [35, 36], Denmark [37], Norway [38] and Japan [39] have all reported comparable incidence and prevalence rates. A meta-analysis of European studies estimated a mean prevalence rate of 0.75% and an incidence rate of 51 cases per 100,000 per year [12].

Higher rates of hyperthyroidism are observed in iodine deficient countries, mostly due to an excess of nodular thyroid disease in the elderly [63, 65], mostly due to an excess of cases of toxic nodular goiters [60].

Table 4 Prevalence of hyperthyroidism in iodine sufficient and iodine deficient countries

Author, country, publication year	Study date	Sample no.	Age (yrs)	Female (%)	Iodine intake (UIC)	Prevalence, %		
						M	F	Total
IODINE SUFFICIENT								
Tunbridge, UK, 1977 [32]	1972-1974	2,779	>18	54	811 nmol/24h	0.2	1.9	1.1
Mogensen, Denmark, 1980[66]	1972-1974	439,756	>0	50	n/a	n/a	n/a	n/a
Berglund, Sweden, 1990[67]	1970-1974	258,000	>0	52	n/a	n/a	n/a	n/a
Konno, Japan, 1993[39]	1990-1991	4,110	Adult	29	n/a	0.3	0.5	0.3
Galofre, Spain, 1994[47]	1990-1992	103,098	15-85	57	n/a	n/a	n/a	n/a
Berglund, Sweden, 1996[35]	1988-1990	231,774	>0	53	n/a	n/a	n/a	n/a
Vanderpump, UK, 1995[34]	1975-1994	1,877	38-93	56	102 µg/g cr	0.2	3.9	2.5
Bjoro, Norway, 2000[38]	1995-1997	94,009	>20	50	n/a	0.1	0.3	0.2
Canaris, USA, 2000[42]	1995	24,337	>18	56	n/a	n/a	n/a	0.1
Hollowell, USA, 2002[31]	1988-1994	13,344	>12		145 µg/l	n/a	n/a	0.2
Volzke, Germany, 2003[48]	1997-2001	3,941	20-79	48	12 µg/dL	n/a	n/a	0.4
Flynn, UK, 2004[43]	1993-1997	369,885	>0		n/a	n/a	n/a	0.6
O' Leary 2006[49]	1981	2,115	16-89	50	2,115	0.1	0.2	0.1
Leese, UK, 2007[52]	1994-2001	388,750	>0	52	n/a	0.2	1.3	0.8
Lucas, Spain, 2010[54]	2002	1,124	18-74	56	150 µg/l	0.2	0.2	0.2
Asvold, Norway, 2012[30]	1995-2008	15,106	>20	67	n/a	n/a	n/a	n/a
Delshad, Iran, 2012 [56]	1999-2005	1,999	>20	61	n/a	n/a	n/a	n/a n/a
Unnikrishnan, India, 2013*[57]	2011	5376	18-100	53.7	n/a	0.6	0.7	0.67

Author, country, publication year	Study date	Sample size (N).	Age (yrs)	Female (%)	Iodine intake UIC	Prevalence, %		
						M	F	Total
Sriphrapradang, Thailand, 2014[58]	2009	2545	≥14	46	n/a	n/a	n/a	0.94
Nystrom, Sweden, 2013[36]	2003-2005	631, 239	>0	n/a	125 µg/l	n/a	n/a	n/a
Valdes, Spain, 2017[44]	2009-2010	4,554	18-93	58	117 µg/l	n/a	n/a	0.4
IODINE DEFICIENT								
Kalk, South Africa, 1981[68]	1974-1984	1,246,294	>15	48	n/a	n/a	n/a	n/a
Aghini-Lombardi, Italy, 1999[60]	1995	992	>15	58	55µg/l	2.9	3.0	2.9
Knudsen, 1999, Denmark[61]	1993-1994	2,613	41-71	49	70µg/l	0	1.2	0.6
Knudsen, 2000, Denmark[37]	1997-1998	2,293	18-65	79	45µg/l	n/a	n/a	0.4
Knudsen, 2000, Denmark[37]	1997-1998	2,067	18-65	79	61µg/l	n/a	n/a	0.8
Hoogendoorn, 2006, Netherlands[62]	2002-2003	5,167	>18	54	n/a	0.2	0.6	0.4
Laurberg, 2006, Denmark[63]	1997-1998	310,124	18-65	50	68µg/l	n/a	n/a	n/a
Laurberg, 2006, Denmark[63]	1997-1998	225,707	18-65	53	53µg/l	n/a	n/a	n/a

Data is for cases of overt hyperthyroidism except where otherwise stated. Iodine status is based on reported status by authors; spaces are left blank where there is no data on incidence or prevalence or where the data is unclear from the report.

*Study from 8 cities with a wide mix of iodine status ranging from sufficient to deficient. Studies in specific population groups such as children, pregnant women, specified co-morbid states, and unstable iodine nutrition are excluded. UIC, urinary iodine concentrations.

Figure 2 Global epidemiology of overt hyperthyroidism

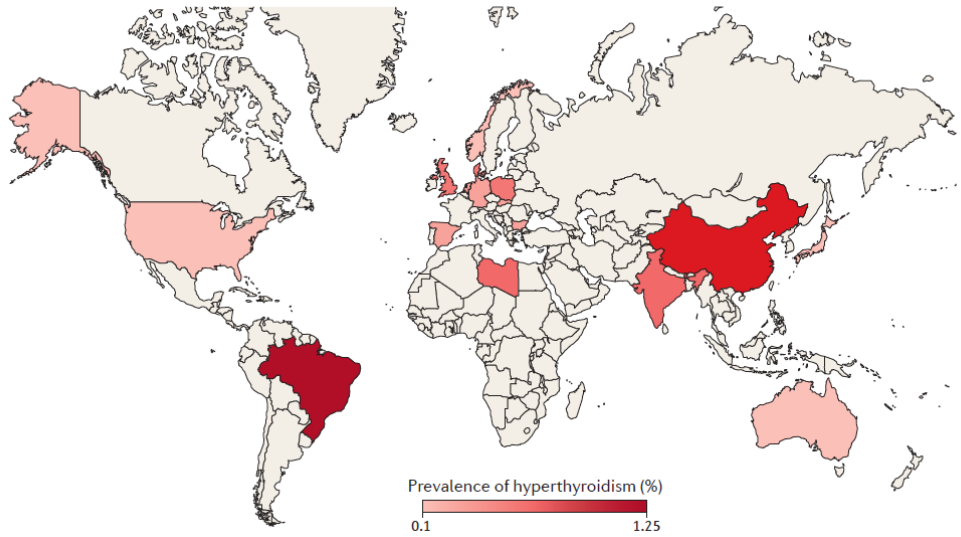


Figure created using Tableau software version 10.3. World map showing global prevalence of hypothyroidism based on epidemiological surveys. If multiple studies have been performed on the prevalence of hyperthyroidism from one country, the median value was used. The deeper the shade of red the higher the prevalence of hyperthyroidism.

1.3 THYROID FUNCTION TESTING AND REFERENCE RANGES

Thyroid status is tightly regulated by the hypothalamus-pituitary-thyroid (HPT) axis [69] (**Figure 3**). Only unbound (free) hormones are biologically active. Clinically, thyroid function is assessed by measuring free thyroxine (FT4), free tri-iodothyronine (FT3) and the pituitary hormone thyrotropin (TSH); the complex inverse relationship between them renders TSH the more sensitive marker of thyroid status [70]. TSH levels are therefore used to ascertain the extent of thyroid dysfunction. Overt hypothyroidism is defined as an elevated TSH and decreased serum levels of FT4 or FT3. Subclinical hypothyroidism (SCH) is defined as an elevated TSH (>5 mU/mL), but normal circulating thyroid hormone levels. In healthy individuals, intra-individual variation in TSH, FT3 and FT4 is less than 50% of inter-individual variation, [71] thyroid parameters are also

stable over time in healthy adults and are largely genetically determined [72]. However, in children cross-sectional studies suggest that FT3 substantially falls and FT_4 rises from age 4, but there are no longitudinal studies to confirm these observations [55, 73]. Studies have also shown that adult reference intervals for thyroid hormone levels are not universally applicable before puberty [74, 75]. Taken together, this raises the possibility that thyroid hormones do not necessarily track over childhood.

Recently there has been increasing interest in viewing thyroid function as a continuous variable in determining risk of adverse outcomes. Studies have shown a "U" shaped curve throughout the normal range with maternal thyroid function and offspring neuro-development [76]. Other studies have shown variation in FT4 to be a continuous risk factor [77] or with increased risk arising well below traditional thresholds for defining abnormal thyroid function [78]. This continuous approach may be more clinical meaningful, particularly when determining who might benefit from treatment rather than a binary cut-off.

Figure 3 Hypothalamus-Pituitary-Thyroid feedback loop

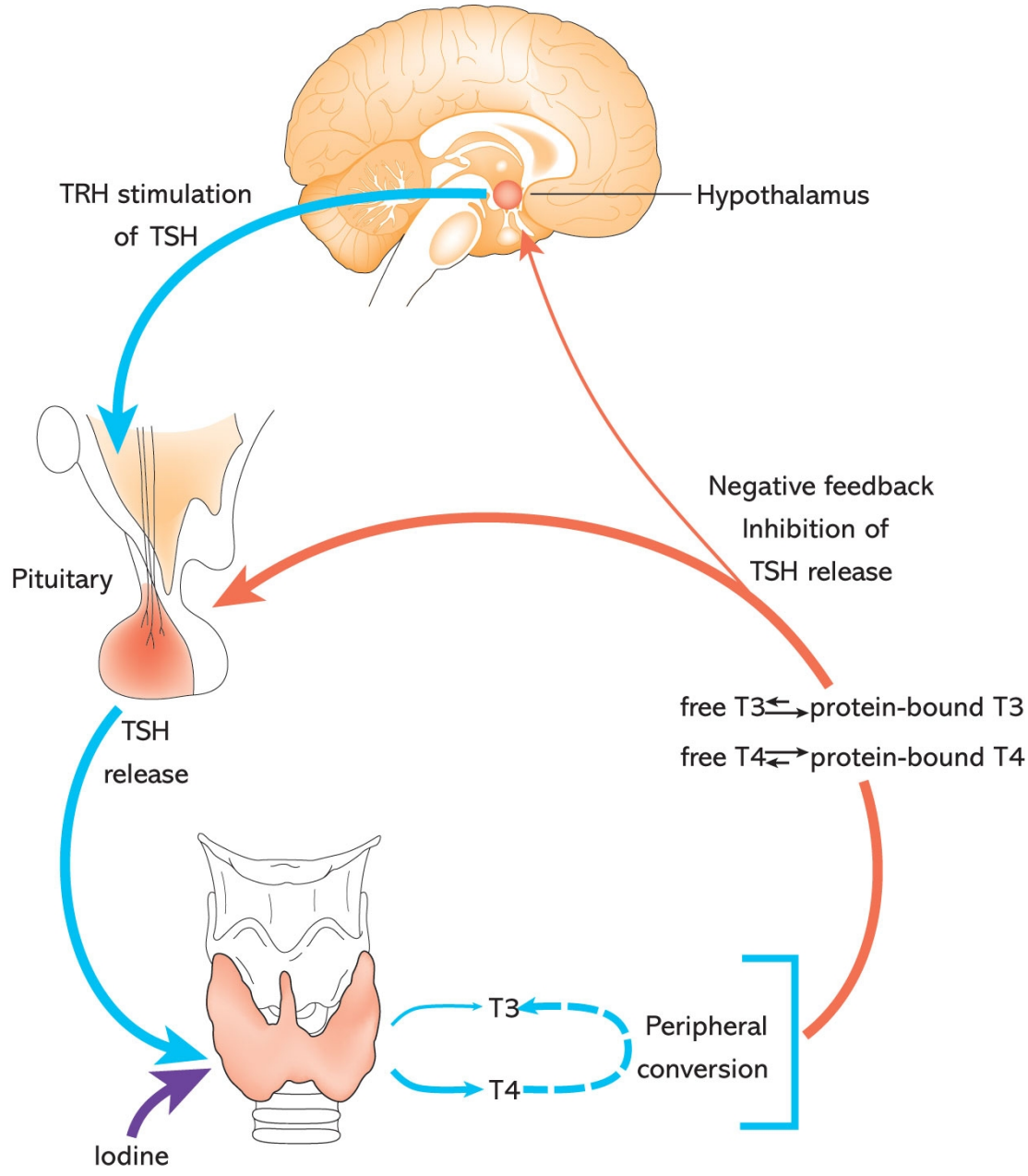


Figure used with permission from Vijay Panicker.

In the last 20 years, there has been a steady increase in thyroid function testing [79] which has resulted in many individuals being identified with overt and subclinical thyroid disease. The reasons behind this widespread thyroid function testing are unclear. This is of increasing clinical

importance as there is current controversy as to appropriate reference ranges for serum thyroid hormone levels with large number of individuals around the borders of the range and we are unsure as to the consequences of this. For instance, the reference range is defined, as two standard deviations above and below the mean in a group of apparently disease-free individuals. However, it has been argued that an epidemiological approach is perhaps more meaningful, defining abnormal values as those associated with adverse consequences [80]. To take this further, one could view TSH and FT4 as continuous measures with optimal sub-optimal and pathological zones.

1.4 RISK BENEFITS OF CORRECTING ABNORMAL THYROID FUNCTION

It is well established that correct of overt hypothyroidism and hyperthyroidism is associated with substantial health benefits [1, 2]. Subclinical thyroid disease is associated with adverse bone outcomes, atrial fibrillation and to a lesser extent, quality of life [3, 81-83]. It is subclinical hypothyroidism and its impact on cardiovascular outcomes that will drive the majority of clinical management decisions as well as determining the cost-effectiveness of detecting and treating subclinical thyroid disease [84].

International guidelines [85] only recommend consideration of levothyroxine therapy at TSH levels less than 10mU/l when there are clear symptoms of hypothyroidism, positive thyroid antibodies or

evidence of atherosclerotic cardiovascular disease or heart failure (evidence level B). However, it is unclear which patients with a TSH between 4.5-10.0mU/l will benefit most [86, 87].

Although awareness of subclinical thyroid disease and its potential for adverse effects on health has substantially increased, the optimal management remains uncertain. This is not due to safety concerns regarding therapeutic options as treatments for subclinical thyroid disease are effective, cheap and easy to monitor. It is only the uncertainty regarding the magnitude of the clinical benefit of treatment that has led to divergent opinions regarding both screening and management [79, 81, 88, 89]. At present data remain lacking due to limited trials in this area with adequate power [3]. This is further compounded because the diagnosis of subclinical thyroid disease is based on an individual having abnormal TSH levels with normal FT3 and FT4 levels and the exact definition of the upper limit of a normal TSH remains contentious [90, 91]. Screening and correction of thyroid disease in the healthy adult population is likely to have an unfavourable cost to benefit ratio. However, pregnancy may be a special situation which would merit screening for and correction of borderline thyroid disease.

1.5 TREATMENT OF HYPOTHYROIDISM AND HYPERTHYROIDISM

In both hypothyroidism and hyperthyroidism, the goal of treatment is to restore well-being and TSH levels to the normal range. In primary hypothyroidism treatment is usually life-long. Levothyroxine (T4) is the

mainstay of treatment and is well tolerated by the majority of patients, however a minority require liothyronine (T3) or desiccated thyroid extract [92]. Treatment for hyperthyroidism does not usually require greater than 18 months therapy with anti-thyroid drugs such as carbimazole or propylthiouracil [1]. However definitive treatment (Radio-iodine or Surgery) may be required and these usually result in individuals becoming hypothyroid and requiring long term treatment with levothyroxine. Again, treatment targets may be best regarded on a continuum rather than a binary in-range, out of range set-point.

1.6 THYROID FUNCTION IN PREGNANCY

Thyroid dysfunction, particularly lower thyroid function is common in women of childbearing age [93]. Furthermore, pregnancy results in additional demands being placed on the thyroid axis these are summarized in **Table 5**. Some women cannot meet this demand, and this results in low thyroid function during pregnancy.

Table 5 Summary of physiological changes during pregnancy and their impact on thyroid function.

Physiological change	Effect on thyroid function test	Key point for clinicians
↑ Thyroxine-binding globulin	↑ Serum total T3 and T4 concentration	Total thyroid hormone levels may be misleading rely on free thyroid hormone levels.
Secretion of human chorionic gonadotrophin	↑ Free T4 and ↓ TSH	High human chorionic gonadotrophin levels may result gestational thyrotoxicosis. This usually only requires symptomatic treatment but needs to be distinguished from pathological thyroid disease.
↑ Iodine clearance	↓Hormone production in iodine deficient areas	Need to be mindful of iodine deficiency and ensure optimal intake ideally prior to conception.
↑ Plasma volume	↑ T3 and T4 pool size	
Increased Type 3 5-deiodinase (inner ring deiodination) activity from the placenta	↑ T3 and T4 degradation	Another cause of increasing thyroid demand in pregnancy.
Thyroid enlargement (in some women)	Increased thyroglobulin	Be aware that a small goitre is common in pregnancy, but may be a sign of low thyroid function so merits thyroid function testing

As a result of these major changes to physiology during pregnancy, gestational thyroid disease is best diagnosed using pregnancy specific reference ranges [4]. Ideally locally derived reference ranges should be

used where possible which take into account additional factors such as the iodine status and ethnicity of the local population [93].

1.6.1 Epidemiology of thyroid disease in pregnancy

Thyroid disease is common in pregnancy and approximately 1-2% of women who are pregnant are established on levothyroxine prior to pregnancy [94]. Overt maternal hypothyroidism - elevated concentrations TSH and low maternal free FT4 occurs in approximately 0.2-0.6% of pregnant women [95, 96], whereas subclinical hypothyroidism (SCH - elevated TSH and normal FT4) can occur in up to 18% of pregnancies depending on the precise definition and TSH cut-point used [4, 93]. Isolated hypothyroxinemia (IH) is defined as a normal TSH with FT4 below the 2.5 percentile was originally considered to be a pregnancy specific condition possibly arising as a consequence of mild iodine deficiency. This concept has been more recently challenged as it occurs in iodine sufficient areas and does not typically resolve with iodine supplementation [97, 98]. Other factors including elevated BMI, older age, iron status and placental angiogenic factors have all been identified as likely risk factors for IH [99-101]. Overt hyperthyroidism, is less common and is usually due to Graves' disease occurs with a frequency of approximately 0.2% [102] however previously treated maternal Graves' disease prior to pregnancy is more common and can occur prior to 1% of pregnancies [102]. New onset pathological hyperthyroidism during pregnancy is much rarer with a prevalence of 0.05% for Graves' disease [102]. Gestational thyrotoxicosis (suppressed TSH and elevated FT4) mainly through excess hCG and usually associated with hyperemesis

gravidarum, occurs in up to 3% of pregnancies [102]. Subclinical hyperthyroidism most commonly occurs as a result of peak hCG levels [103] although may occur due to pathological thyroid disease. Owing to this dual cause of subclinical hyperthyroidism its true consequences and prevalence are poorly studied.

1.6.2 Consequences of maternal thyroid dysfunction in pregnancy

Overt hypothyroidism has been repeatedly associated with a higher risk of adverse obstetric outcomes including foetal loss, premature delivery, low birthweight and pre-eclampisa [95, 104]. Effects have been observed on foetal neuro-development, a large case-control study demonstrated children born to women with untreated hypothyroidism had a 7 point lower IQ than women with normal thyroid function [105]. SCH is also associated with similar adverse obstetric outcomes as overt hypothyroidism, albeit with a more modest effect. Studies have demonstrated an increased incidence of adverse pregnancy outcomes including preterm delivery, placental abruption, respiratory distress, early pregnancy loss and admissions to the intensive care unit [106-110] but it has not been associated with impaired development of offspring.

Although IH is also regarded as a mild form of thyroid failure it is associated with offspring developmental outcomes [76] but not obstetric outcomes in stark contrast to SCH. Intriguingly, the relationship between maternal FT4 and offspring IQ appears to be “U” shaped with individuals with hypothyroxinemia having lower IQ, and lower grey matter and

cortical volume [76]. Maternal IH has also been shown to increase the risk of autism [111].

TPO antibody positivity is a major risk factor for SCH [112]. However, the combination of SCH and TPO antibody positivity appears to have a synergistically adverse outcome. In particular, adverse synergistic associations occur for miscarriage, premature delivery and gestational diabetes mellitus [113]. It also appears to be a risk factor in its own right for miscarriage and pre-term delivery [114]. TPO positivity may also impair thyroidal response to hCG [103] and result in more profound hypothyroidism due to unmet pregnancy demands.

Whether or not all pregnant women should be screened for thyroid disease remains controversial. This is despite thyroid dysfunction being common and often asymptomatic in women of child-bearing age with substantial adverse implications for foetal and maternal wellbeing [4, 95, 115].

Data are less clear as to whether treatment of thyroid disease initiated during pregnancy results in clear benefits. Whilst data are compelling for correction of overt thyroid disease [105] at present the benefits of treating SCH and IH in pregnancy remain unclear. Two large prospective intervention trials demonstrated no impact on offspring IQ [116, 117], and one of these also failed to identify benefits on obstetric outcomes [118] and one prospective trial demonstrated a decrease in the composite

number of adverse pregnancy/neonatal outcomes in thyroid peroxidase antibody positive women with subclinical hypothyroidism [110].

1.7 AIM OF THIS PhD THESIS

Although it is widely accepted that pathological variation in thyroid hormone levels has major adverse outcomes on both childhood development and adult health it is less clear whether more minor variation in thyroid function – including variation across and just outside the reference range – has sufficient impact on key health outcomes to justify intervention with thyroid hormone supplementation in selected individuals. Assessing the magnitude of this potential benefit will be a key component of this thesis. Given that pregnancy places additional demands on the thyroid and requires tighter control, this will be a key focus of my work. The impact of current practice of thyroid hormone prescribing and potential impact of screening for low thyroid function in pregnancy will also be explored.

The thesis will comprise of the following research chapters

1) Analysis of the effects of variation in thyroid status within the population reference range on key cardiovascular, metabolic and bone outcomes.
2) Assessment of the effect of variation in thyroid status on body composition in children.
3) Assessing trends in UK management of primary hypothyroidism.
4) Assessing the quality of levothyroxine prescribing in pregnancy and the effects on obstetric outcomes.
5) Analysis of the potential obstetric benefits of screening for and treating low thyroid function in pregnancy.

Chapter 2 Methods

In this chapter I summarize the various cohorts and datasets used in my PhD analyses. Further details of the phenotypes, biochemical measures and statistical analyses used are described in the relevant chapters.

2.1 COHORTS AND DATASETS USED IN THIS THESIS.

2.1.1 The Avon Longitudinal Study of Parents and Children (ALSPAC)

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a geographically based birth cohort that recruited pregnant women residing in Avon, UK, with an expected delivery date between 1st April 1991 and 31st December 1992. 14,541 pregnant women were initially enrolled with 14,062 children born; this was increased to 14,633 by recruiting children at age 7 who were initially eligible, but not recruited. Approximately 80–90% of the eligible population was recruited. Participants have been extensively followed from the 8th gestational week using a combination of self-reported questionnaires, medical records and physical examinations. This cohort is described in detail elsewhere (http://www.alspac.bris.ac.uk)[119, 120].

2.1.2 The General Practice Research Database (GPRD)

The GPRD (now called the Clinical Practice Research Datalink www.CPRD.com) has been well described previously [121]. It is currently the largest computerized database of anonymized medical records from primary care that is linked with other healthcare data.

The CPRD contains computerized medical records of over 5,000,000 people from 508 primary care practices throughout the UK. The CPRD has been validated for use in research on disease epidemiology, drug safety and adverse drug reactions [122-124]. In particular, data on drug exposure and diagnoses [125] are of high quality [126]. It is also representative of the overall UK population with regard to age and sex [127, 128]. Few practices opt out of the CPRD system so this is unlikely to cause bias in the dataset [127].

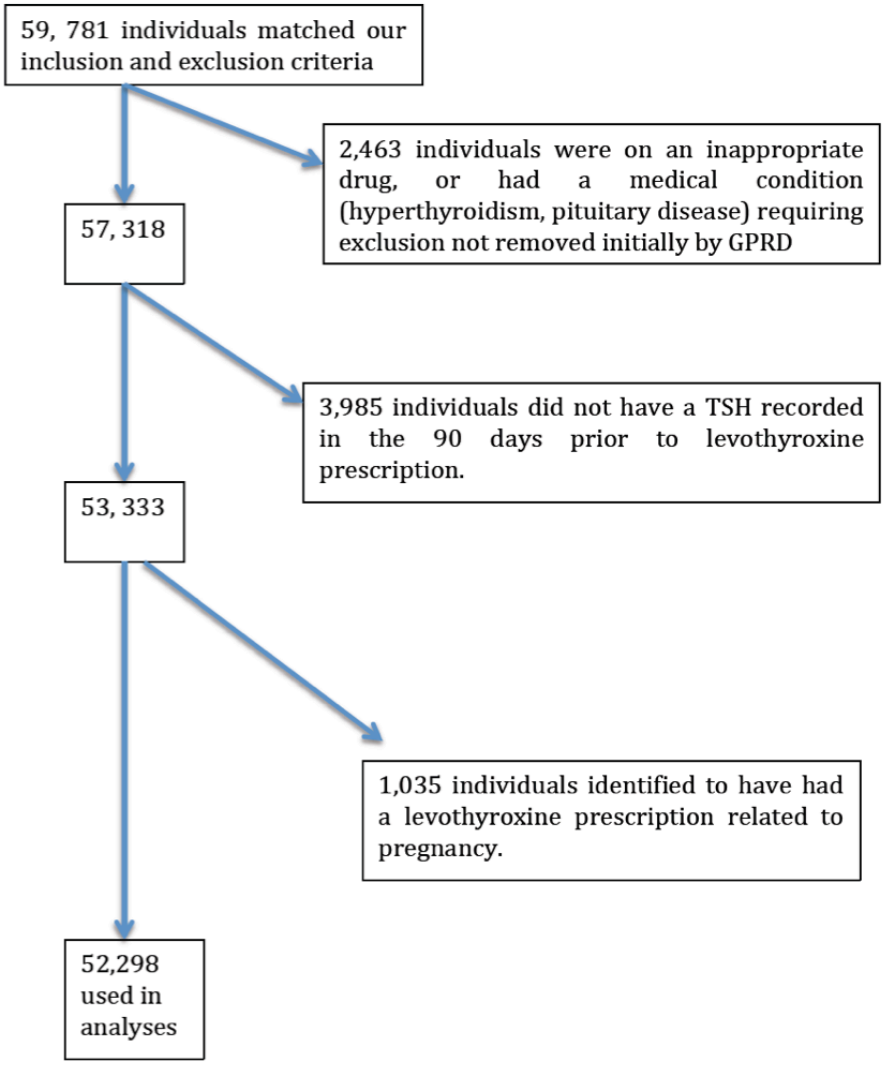
CPRD contains detailed clinical information on diagnoses, patient symptoms, laboratory results, drug prescriptions and hospital referrals [127]. These are identified through read codes. Validation studies have confirmed the high quality and accuracy of studies undertaken using CPRD [125].

THE CPRD dataset used for this work consisted of patients aged between 18-99 years at their first-ever (index) prescription of levothyroxine which occurred between 01/01/2001 - 30/10/2009. Patients also had to have at least 12 months of up-to-standard (data that met GPRD quality standards) follow-up prior to their index levothyroxine. Patients with a prescription

record at any time on the database of amiodarone, carbimazole, propylthiouracil, lithium, interferon, thalidomide or sunitinib were excluded; as were patients previously diagnosed with or treated for hyperthyroidism prior to their index levothyroxine as evidenced by medical codes and referral codes in the GPRD relating to Graves' disease, thyrotoxicosis, hyperthyroidism, toxic multi-nodular goiter, thyroidectomy and radio-iodine. Patients with a documented diagnosis of thyroid cancer or a diagnosis of pituitary disease or pituitary surgery were also excluded.

In all, 59,781 individuals matched our initial inclusion criteria 57,318 individuals matching our study inclusion criteria were included of whom 53,333 (93.0%) had a prescription within 90 days after a documented TSH level. 1,035 individuals were classified as having received levothyroxine related to pregnancy and were analyzed separately (**Figure 4**).

Figure 4 Flow of individuals studied in CPRD



2.1.3 Controlled Antenatal Thyroid Screening Study (CATS)

The Controlled Antenatal Thyroid Screening study was a large randomised controlled trial to investigate the benefits of screening treating low maternal thyroid function on offspring IQ. In this study, pregnant women were invited to participate at their first antenatal hospital visit. The women were recruited from 10 centres in the United Kingdom (Bristol, Glan Clwyd, Llandough, Neville Hall, Princess of Wales, Royal Glamorgan Hospital, Royal Gwent, Singleton and Morriston, University Hospital of Wales and Wrexham) and 1 center in Italy (Turin). Exclusion criteria were an age of less than 18 years, a gestational age of more than 15 weeks 6 days, twin pregnancies, and known thyroid disease. Approval of the study was obtained from research ethics committees in the United Kingdom and Italy, and all participants provided written informed consent.

Blood samples were sent to the laboratory at the University Hospital of Wales, Cardiff (for UK centres), or to Ospedale Sant'Anna, Turin, Italy, for measurement of TSH and FT4 levels. On receipt of samples, women were randomly assigned with the use of a computer-generated block design to the screening or control group.

Serum samples from the screening group were immediately assayed for levels of TSH and FT4. Serum samples from women in the control group, stored at −40°C, were assayed for levels of TSH and FT4 after delivery. Women were classified as positive if they had TSH in the highest 2.5 % and or FT4 in the lowest 2.5%. They were treated with levothyroxine (proposed starting dose, 150 mcg per day). Levels of TSH and free T_4 were

checked 6 weeks after the start of levothyroxine therapy and at 30 weeks' gestation, with adjustment of the dose as necessary. The target thyrotropin level was 0.1 to 1.0 mIU/l.

Women in the screening and control groups who had positive test results received standard routine care and were advised to visit their family physician after delivery to determine whether levothyroxine therapy should be continued or initiated, respectively.

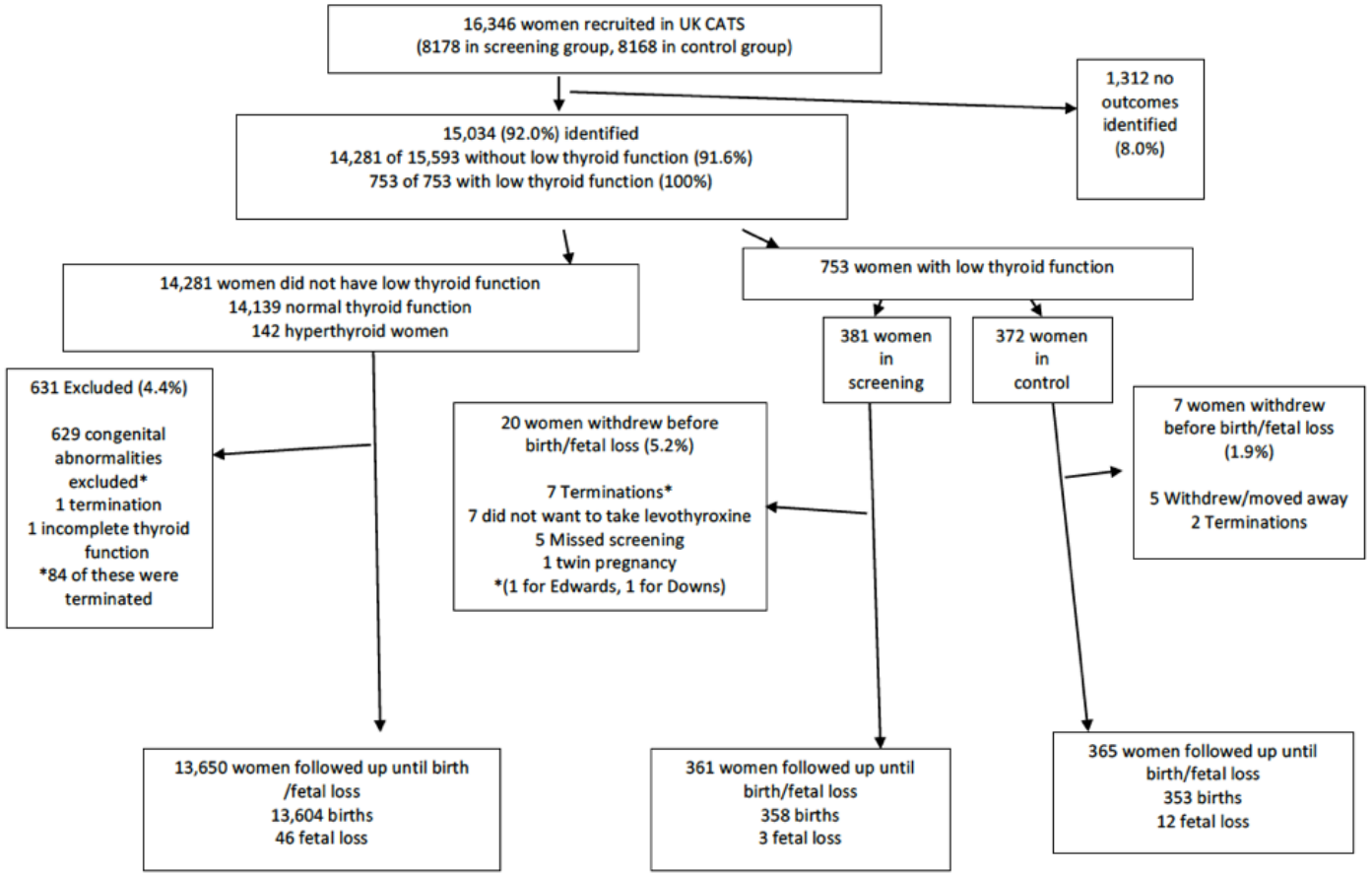
In the United Kingdom, levels of serum TSH and free T_4 were measured using immunochemiluminescence (ADVIA Centaur, Siemens Healthcare Diagnostics). The 95% range of TSH was 0.15 to 3.65 mIU/l, and the 95% range of free T_4 levels was 8.4 to 14.6 pmol/l.

The study recruited 21, 846 women (16,346 from the 10 centres in the UK) and 5,500 from Turin in Italy (although women recruited in Italy were not used in my analysis). To summarize the original CATS study 10,924 women were assigned to the screening group and 10,922 were assigned to the control group. 4.6% of women were defined as having a positive screening result in the screening group and 5.0% had a positive screening result in the control group. In both groups, similar proportions were classified as having a high TSH or low FT4. Approximately 5% of women in each of the groups had both a high TSH and a low FT4. There was no difference between the treatment groups in the baseline characteristics (gestational age at screening, maternal weight, maternal smoking, age

when mother left full time education, maternal age at delivery paternal age at delivery, % male children). The only substantial difference due between groups is that TSH levels were slightly higher in the screening group than the control group (Median 3.8 mU/l IQR 1.5-4.7) vs (3.2 mU/l IQR 1.2-4.2). The IQ of the children was assessed at age 3 in the original study and then again in an additional study at age 9 [129].

Detailed obstetric data was not collected in the original CATS study, however some records were kept on some patients with abnormal thyroid function regarding whether there was a miscarriage or a stillbirth or whether there was a termination. As data was not routinely collected analysis of outcomes were limited in the original study. A summary of the participants used in my analysis in this thesis is shown in **Figure 5**.

Figure 5 Flow diagram of the CATS participants used in my analyses



2.1.4 Secure anonymised information linkage (SAIL)

The SAIL databank is held and managed within the Health Information Research Unit at Swansea University and contains health social and education data on three million residents of Wales [130]. The demographic data comprises the commonly-recognised person-based variables of first name, surname, sex, date of birth, postcode and NHS/hospital number. Matching records in SAIL has been demonstrated to be highly sensitive and specific [130]. The clinical data covers data such as diagnostic tests, therapeutic procedures and interventions. The clinical data used in this analysis was from the Patient Episode Database for Wales (PEDW)[130]. PEDW is a register of all clinic and inpatient activity undertaken in Welsh NHS hospitals and processes over a million hospital episodes annually including information on diagnoses, admissions, hospital births, and surgical operations including Caesarean sections. This data was linked to key trial data from the CATS study to enable us to explore obstetric outcomes in trial participants.

Chapter 3 A meta-analysis of the consequences of variation in thyroid function across the population reference-range using data from population based birth cohorts

In the introduction to this thesis I highlighted the importance of thyroid hormone and the clinical consequences of overt thyroid disease. In order to appraise the impact of more modest variation of thyroid status I undertook a meta-analysis of studies using population based cohorts which assessed the effect of variation in thyroid status within the population reference-range on key health outcomes. This approach enabled me to explore both the magnitude and consistency of these effects.

3.1 INTRODUCTION

As we have already seen thyroid disease is common and its treatment is simple, inexpensive with well-established safety data [1, 2]. It is therefore essential to determine the consequences and the potential benefits of correcting subclinical thyroid disease [3].

Increased use of thyroid function testing [79] has resulted in many individuals being identified with subclinical thyroid disease. The prevalence of subclinical hypothyroidism is between 4-8.5% [31, 42] rising to 15% in elderly populations [3, 131]. Subclinical hyperthyroidism is less common with a prevalence of 1-5% in the elderly [132]. Treatments for subclinical thyroid disease are effective, cheap and easy to monitor; it is

the uncertainty regarding the magnitude of the clinical benefit of treatment that has led to divergent opinions regarding screening and management [79, 81, 88, 89]. Data are lacking due to limited trials in this area with adequate power [3]. This is further compounded because the diagnosis of subclinical thyroid disease is defined on having abnormal thyroid stimulating hormone (TSH) levels with normal free tri-iodothyronine (FT3) and free thyroxine levels (FT4) and the exact definition of the upper limit of a normal TSH remains contentious [90, 91].

Whilst subclinical thyroid disease is robustly associated with adverse bone outcomes, atrial fibrillation and to a lesser extent, quality of life [3, 81-83], it is subclinical hypothyroidism and its impact on cardiovascular outcomes that will drive the majority of clinical management decisions as well as cost-effectiveness considerations for its detection and treatment [84]. A meta-analysis [87] identified that subclinical thyroid disease might be associated with adverse coronary heart disease and mortality outcomes; although the point estimates for the relative risk for coronary heart disease extended below equality. Limiting analyses to studies with the more robust methodologies and lower risk of selection bias decreased risk estimates. Whilst this meta-analysis [87] was unable to confirm a positive association between subclinical thyroid disease and coronary heart disease and mortality in the general population; it did indicate that the negative impact of sub-clinical hypothyroidism may be more substantial in younger individuals RR=1.51 (95%CI 1.09-2.09).

American Thyroid Association guidelines [85] recommend consideration of levothyroxine therapy at TSH levels less than 10mU/l when there are clear symptoms of hypothyroidism, positive thyroid antibodies or evidence of atherosclerotic cardiovascular disease or heart failure (evidence level B), but it is unclear which patients with a TSH between 4.5-10.0mU/l will benefit most [86, 87].

Analyzing cohorts of individuals identified to have subclinical thyroid disease has key limitations, due to small study size and potential for substantial selection bias. This selection bias arises, as subclinical thyroid disease is often asymptomatic and individuals have their thyroid function measured for a variety of reasons, including screening in patients with diabetes, therefore individuals who have their thyroid function measured are not representative of the general population.

An alternative approach is to study the phenotypic consequences of variation in thyroid hormone parameters within the general population. Studies here are considerably larger and less prone to selection bias than any available in subclinical thyroid disease. Given the consequences of overt thyroid disease are well established [133, 134] it would then be possible to make assumptions of the consequences of subclinical thyroid disease if the nature of the effects of variation within the reference range and the effects of overt thyroid disease were concordant. This review will therefore highlight the phenotypic consequences of modest variation in thyroid function within the population reference-range.

I therefore undertook a review and meta-analysis of the effects of variation in thyroid status within the population reference range on cardiovascular, metabolic and bone outcomes.

3.2 METHODS AND DATA SYNTHESIS

Combinations of 'TSH', 'FT4', 'FT3', 'thyroid function', 'blood pressure', 'cholesterol', 'lipid levels', 'cardiovascular disease', 'myocardial infarction' 'arrhythmia' 'atrial fibrillation' 'stroke' 'bone mineral density', 'osteoporosis', 'osteopenia', 'peak bone mass' 'fracture' 'BMI' 'weight' 'metabolic syndrome' 'ATP-III' 'pregnancy' 'cancer' 'neurological development' 'mood' 'behavior' 'depression' 'anxiety' 'neurological' separately and in conjunction with the terms 'reference-range' and 'normal range' up to September 2013 were used to search MEDLINE via an Ovid Server and the Cochrane database. The references of retrieved papers were also reviewed. Only English-language papers were studied.

The nature of this review, limited the use of the GRADE scoring criteria [135] as all our studies were observational. However, the GRADE criteria for decreasing and increasing evidence levels was used when appraising papers. Evidence quality was regarded as good if derived from several consistent studies from large epidemiological cohorts with adjustment for important confounders. Evidence quality was regarded as moderate if the number of papers on a topic were limited, or studies were

conflicting, but still from good data sources, finally evidence was regarded as poor if it was derived from studies with imprecise or sparse data or with a high probability of reporting bias.

I then undertook an inverse-variance, fixed-effects weighted meta-analysis, to demonstrate the odds of developing adverse outcomes in individuals with TSH levels in the upper part (upper third) of the reference-range versus those in the lower part (lower third) of the reference-range for cardiovascular, metabolic and bone outcomes. Analyses were then repeated using a random-effects meta-analysis as it is not unreasonable to assume different effects for different aspects of cardiovascular metabolic and bone outcomes. Additional analysis was done for neuropsychological outcomes but this was not included in the meta-analysis due to the lower quality of data.

Salman Razvi and Simon Pearce from Newcastle University acted as second readers and were in complete agreement with me for papers to be included.

3.3 RESULTS

A total of 985 English-language papers were reviewed; studies analysing associations in thyroid hormone parameters outside the reference-range, editorials, individual case studies were excluded. 40 papers were found to be suitable, no published papers studying variation in thyroid function within the reference-range were found to be unsuitable. Information

related to authorship, year of publication, number of subjects, study design, and results were extracted and formed the basis for the report.

Overall the evidence base was consistent and of good quality for cardiovascular and metabolic outcomes, was of moderate quality for metabolic and pregnancy outcomes and was of poor quality for neuropsychological outcomes. An overview of the phenotypic associations of variation in thyroid function within the reference-range is shown in **Table 6.**

Table 6 Summary table of the associations between variation in thyroid hormone parameters within the population reference range and key phenotypic outcomes

Outcome	Association	Parameter	Studies	Evidence quality
Blood Pressure	Yes	TSH	[136-138]	Good
Cholesterol and lipid levels	Yes	TSH	[139, 140]	Good
Cardiovascular mortality	Possible	TSH	[141, 142]	Moderate
BMI	Yes	TSH	[143-146]	Good
Metabolic syndrome	Yes	TSH	[140, 147]	Moderate
Pregnancy outcomes	Yes	TSH	[148]	Moderate
BMD	Probable	TSH	[149-154]	Good in females. Moderate in males.
BMD	Probable	FT3 FT4	[155, 156]	Moderate
Depression	Unclear	TSH	[157]	Poor

3.3.1 The effect of variation in thyroid status within the population reference range on cardiovascular outcomes

Cardiovascular outcomes are summarized in **Table 7**. There is growing evidence that higher levels of TSH are associated with worsening blood pressure [136-138] and also lipid levels [139, 140]. Furthermore these associations are also present in children [138] highlighting that TSH influences cardiovascular risk factors throughout life. From these studies, it appears that variation in TSH levels is associated with a change in both systolic and diastolic blood pressure, with approximately a 2mmHg increase per 1mU/l rise in TSH. Whilst a modest effect; over the reference-range this difference is equivalent to between 33-50% of the blood pressure change observed with anti-hypertensive monotherapy [158].

The impact of variation in TSH over the reference on lipid levels is more modest with a change in total cholesterol of only 0.12-0.20mmol/l between the upper and lower part of the reference-range [139]. This beneficial impact of thyroid hormone on lipids may have become inflated in current prescribing practice as modest dyslipidemia was found to be a major motivator in prescribing levothyroxine for borderline thyroid function [159].

Analysis in the Nord-Trøndelag Health Study (HUNT Study), identified that higher TSH levels within the reference-range were associated with higher

mortality from coronary heart disease in females [141]. This is in keeping with the observed negative impact of rising TSH on blood pressure and lipid levels. Compared to women with a TSH level in the lower third of the reference-range the hazard ratios for coronary heart disease mortality were 1.41 (95%CI 1.02-1.96) and 1.69 (95%CI 1.14-2.52) for women in the middle and higher third respectively [141]. After adjusting for age and smoking the hazard ratio for a 1.0mU/l rise in TSH was 1.37 (95%CI 1.12–1.68). Intriguingly the association between TSH and cardiovascular mortality appears to be mediated by components other than lipids and blood pressure as adjusting for age, smoking, serum creatinine, cholesterol, use of hypertensives, systolic blood pressure and diastolic blood pressure resulted in only a modest fall in the hazard ratio for a 1.0mU/l rise in TSH to 1.30 (95%CI 1.06–1.60) although residual confounding remains a possibility.

The lack of an observed association between TSH and cardiovascular mortality in males may be due to insufficient power as there were over twice the number of females in this study than males; however, there was weak evidence of interaction by sex. Notably extending the observation period for a further 4 years [142] identified that the association between TSH and mortality from coronary heart disease in women remained similar over the increased follow-up time and also demonstrated stronger evidence of interaction by sex. This study also identified that compared to women with a TSH level in the lower third of the reference range the risk of mortality from coronary heart disease was

higher in women with subclinical hypothyroidism HR=1.76; (95%CI 1.21, 2.56) or subclinical hyperthyroidism HR=2.29; (95%CI 1.27, 4.13). This important observation demonstrates that the relationship between TSH and mortality heart disease is likely “u” shaped and highlights that if individuals are excessively treated with levothyroxine for subclinical hypothyroidism which results in a suppressed TSH then any benefit on cardiovascular mortality may be lost and mortality even potentially increased. There is also evidence of a similar “u” shaped association with TSH for both cardiovascular outcomes and fracture incidence in individuals on levothyroxine [160].

Although positive associations between TSH levels and cardiovascular risk factors and cardiovascular mortality [142] have been identified there was no evidence of association between TSH levels and risk of being hospitalized with a first myocardial infarction (MI). This finding therefore does not confirm the suggestion that low thyroid function within the reference-range is associated with an increased risk of MI. This finding is difficult to reconcile with the above observations, it may simply be due to lack of power, but another possible explanation is that higher TSH levels within the reference-range may increase the risk of heart disease but this is mechanistically distinct from a typical MI, for example silent MI or diastolic dysfunction. This hypothesis is supported by a large meta-analysis showing an increased risk of heart failure in individuals with subclinical hypothyroidism [161] although evidence for a substantial

impact of TSH variation within the population on heart failure remains limited at present.

Overall these studies demonstrate that higher TSH levels within the population reference-range is associated with worse cardiovascular risk factors and higher mortality, data remain limited and are conflicting for FT4 and FT3 levels [162-164]. It is noteworthy that further studies are required for other important health outcomes in particular stroke. This is particularly relevant as higher thyroid hormone levels are associated with atrial fibrillation [133, 160] a key stroke risk factor.

Table 7 The effect of variation in thyroid status within the population reference range on cardiovascular outcomes

Report	Study Type	Outcome	Comments
Iqbal Norway 2006 [136]	Cross-sectional analysis of a population cohort, the 5th Tromsø study (N=5,872 M:F 1:1.24)	Blood pressure	Variation in TSH within the population reference range was positively associated with blood pressure.
Asvold Norway 2007 [137]	Cross-sectional analysis of a sub-group of the HUNT study, a population cohort (N=27,786) M:F 1:1.84)	Blood pressure	There was a linear increase in systolic blood pressure over the population reference range, for TSH.
Asvold Norway 2007 [139]	Cross-sectional analysis of a sub-group of the HUNT study a population cohort (N=34,851 M:F 1:1.90)	Cholesterol and lipid levels	With increasing TSH levels within the reference range there was linear increase in concentrations of total serum cholesterol, LDL cholesterol, non-HDL cholesterol and triglycerides
Asvold Norway 2008 [141]	Sub-group of HUNT study a population cohort (N= 25,313 M:F 1:2.16) 410 participants had died from coronary heart disease during a median of 8.3 years of follow-up	Cardiovascular mortality	The hazard ratio for cardiovascular mortality in females with a TSH in the upper third of the reference range compared to the lower third was 1.69 (95%CI 1.14 - 2.52), p for trend = 0.005 after adjusting for age, sex and smoking status. No clear association was observed in men.

Lee Korea 2011 [140]	Cross-sectional study from individuals attending clinic (N=7,270 M:F 1:0.74))	Cholesterol and lipid levels	TSH showed modest but statistically significant positive associations with serum total cholesterol, triglyceride, and low density lipoprotein cholesterol (p<0.001).
Itterman Germany 2012 [138]	KiGGS study of children and adoloescents (N= 6,435 children M:F 1:0.94, N=5,918 adolescents M:F 1:0.94)	Blood pressure	Serum TSH levels were associated with hypertension in children OR=1.12 (95%CI 1.00-1.25; p = 0.05) and adolescents OR = 1.19 (95%CI 1.12-1.26 p=< 0.001)
Debeij Norway 2012 [165]	Nested case control in HUNT 2 Cohort (N=515 cases, 1476 controls)	Venous thrombosis	In individuals with FT4 levels above the 98th percentile of the reference range (17.3 pmol/l), the odds of venous thrombosis within one year compared to individuals with FT4 levels below this level were 2.5 (95%CI 1.3-5.0) For TSH the relation was inverse and less pronounced.
Asvold Norway 2012 [142]	12 year follow-up in the HUNT cohort (N=26,707 M: F 1:2.10) 558 participants had died from coronary heart disease during a median of 12.3 years of follow-up 960 participants had been	Cardiovascular mortality, hospitalisation from myocardial infarction	The risk of mortality from coronary heart disease was higher in women with TSH in the middle (HR = 1.41; 95%CI 1.06 -1.87) and upper (HR =1.45; 95%CI 1.01 - 2.08) thirds of the reference range compared to women with TSH in the lower third (p for trend = 0.005). The risk of mortality from coronary heart disease in women with subclinical

	hospitalized with first-time acute myocardial infarction during 12.2 years (median) of follow-up		hypothyroidism (HR = 1.76; 95%CI 1.21 - 2.56) or subclinical hyperthyroidism (HR = 2.29; 95%CI 1.27 - 4.13) was also higher compared to women with TSH in the lower third of the reference range
Iida Japan 2012 [162]	Patients with hypertension and normal thyroid function (N=293 M:F 1:0.96)	Left ventricular mass	Both FT3 (β =0.13) and FT4 (β =0.13) were positively associated with left ventricular mass whereas TSH was negatively associated (β = -0.15)
Kim Korea 2012 [163]	Cross-sectional analysis of hospital clinic attenders (N = 669 M: F 1:0.71)	Coronary artery calcium scores measured by CT	FT4 levels were inversely associated (β=-0.823, p=0.032), with coronary artery calcification in euthyroid healthy subjects. No association was observed with TSH.
Ertas Turkey 2012 [164]	Cross-sectional analysis of consecutive patients attending coronary angiography (N=119 M:F 1:0.55)	Presence of coronary artery disease	Lower FT3 levels within the reference range were associated with increased odds for both the presence and severity of coronary artery disease.

3.3.2 The effect of variation in thyroid status within the population reference range on metabolic outcomes

A summary of the effect of variation in TSH and thyroid hormone parameters within the population reference-range on metabolic outcomes is shown in **Table 8**. Associations have been identified with weight and BMI, metabolic syndrome and glomerular filtration rate (GFR).

Over the past decade cross-sectional studies, mainly in adults, have shown that variation in thyroid status across the population reference-range is associated with substantial differences in body mass index and body composition [143, 145, 146]. Rising TSH levels in an individual are associated with increased weight gain [144, 145]. Baseline TSH may also be associated with weight gain over time, although this may not be apparent for several years [143]. In contrast a reciprocal effect was observed for FT4 which was strongly negatively associated with BMI [143].

Cross–sectional analysis in cohort studies highlighted that the odds of metabolic syndrome as defined by the ATP-III criteria are positively associated with higher TSH levels within the reference-range [147, 166, 167]. It should be highlighted that this association in particular may be due to reverse causation through the impact of the metabolic syndrome on the hypothalamic-pituitary-thyroid axis. Therefore, prospective cohort studies with serial measurements of both thyroid function and metabolic properties assessing the changes in both thyroid hormone status and ATP-III score over time are required.

Variation in TSH within the population reference-range was positively associated with changes in eGFR and also a higher prevalence of chronic kidney disease [168]. Furthermore, the strength of this association was magnified over the subclinical and overt hypothyroid range. The association between TSH and GFR was approximately the same in TPO

positive and TPO negative individuals indicating that immunological processes are unlikely to explain this association. It has been previously observed that GFR increases following T_4 treatment for hypothyroidism [169, 170] and decreases after treatment of hyperthyroidism [169] or after withdrawal of T_4 therapy indicating that variation in thyroid hormone status drives this association. This association between GFR and thyroid status may be explained, at least partially by a diminished ability to excrete free water [171, 172] in hypothyroidism which may result in subsequent changes in volume status.

Table 8 The effect of variation in thyroid function within the population reference range on metabolic outcomes

Report	Study	Outcome	Comments
Knudsen Denmark 2005 [143]	Cross-sectional analysis of DANTHYR study in individuals without thyroid disease (N=4,082)	BMI	A positive association was identified between serum TSH and BMI with a negative association between serum FT4 and BMI. No association was observed between serum FT3 and BMI.
Fox USA 2008 [145]	Framingham Offspring Study. (N=2,407 M:F 1:0.87)	Change in weight over follow-up	Baseline TSH concentrations were not associated with weight change during follow-up. However, an increase in TSH concentration at follow-up was positively associated with weight gain in women
Asvold Norway 2009 [146]	Subgroup of the HUNT population cohort (N=27,097 M:F 1:1.96)	BMI	Variation in TSH within the reference range was positively associated with BMI.
Ruhla Germany 2010 [147]	Metabolic Syndrome Berlin Potsdam (MeSyBePo) cohort (N=1,333 M: F 1:1.77)	ATP III criteria of the metabolic syndrome	Individuals with TSH in the upper normal range (2.5-4.5 mU/l) had increased odds of fulfilling the ATP III criteria of the metabolic syndrome.
Kim Korea 2011 [140]	Cross-sectional study from individuals attending clinic (N=7,270 M:F 1:0.74)	ATP III criteria of the metabolic syndrome	Individuals with high-normal TSH levels had an almost two-fold higher odds of metabolic syndrome compared to those within the lower part of the reference range.
Asvold Norway 2011	Cross-sectional analysis of a sub-group of	GFR	High TSH within the reference range was associated with reduced

[168]	the HUNT population cohort (N=29,480 M:F 1:2.02)		eGFR, although the effect was modest. This trend continued through the sub-clinical range and into overt hypothyroidism.
Svare Norway 2011 [144]	Prospective data from the HUNT 2 and HUNT 3 population cohort studies (N=15,020: M:F 1:1.97)	Weight/BMI	For each 1.0 mU/l increase in TSH amongst women, weight increased by 0.99 kg and BMI increased by 0.3 kg/m2. In men, for each 1.0 mU/l increase in TSH, weight increased by 0.8 Kg and BMI increased by 0.2 kg/m^2
Bassols 2011 [167]	Cross-sectional analysis of a cohort of healthy euthyroid pregnant women at 24-28 weeks gestation. (N=321)	Hba1c, HMW adiponectin, placental weight	Low normal serum FT4 was associated with adverse metabolic parameters.
Prats-Puig Spain 2012 [166]	Cross-sectional study of children attending primary care clinics. (N=234: M:F 1:1.07)	HMW adiponectin, HOMA (IR), visceral fat	Pre-pubertal girls with low-normal FT4 levels have a more dysmetabolic phenotype. S

BMI = body mass index, TSH = thyroid stimulating hormone, HOMA(IR) = insulin resistance

3.3.3 The effect of variation in thyroid status within the population reference range on bone phenotypes and fracture risk

Data are summarized in **Table 9**. Cross-sectional analyses have identified that lower levels of TSH and higher levels of thyroid hormone within the population reference-range are associated with an increased risk of osteoporosis [149-151, 156, 173] and fracture [152, 156]. Data from these studies are largely from healthy post-menopausal women; although this represents the group at greatest risk, generalizability is limited. Even modest variation of 1 unit in TSH and thyroid hormone levels were associated with a substantial change in the odds of osteoporosis and fracture. There may however be a "threshold effect" as the prevalence of vertebral fracture was only substantially increased in individuals with a TSH lower than 1.0 mU/l [152]. In keeping with these findings, greater bone loss occurs in levothyroxine treated patients with suppressed TSH levels than in those without suppression [160, 174]. This reinforces that the potential advantages of treating subclinical hypothyroidism may be lost if patients develop high-normal or subclinical hyperthyroidism through over-replacement.

Data from these studies also highlighted that low levels of TSH, independent of thyroid hormone levels, may have an adverse effect on bone [150, 152] even in younger individuals [155]. This is particularly relevant as peak bone mass determines the structural strength of bone in later life [175] and is a major determinant of an individual's risk of osteoporosis and fracture. The relationship between FT3 and fracture may be more complex than previously believed as FT3 was strongly

positively associated with handgrip and balance [156] key protective factors in determining an individual's falls risk.

Table 9 The effect of variation in thyroid status within the population reference range on bone outcomes

Report	Study Type	Outcome	Comments
Kim Korea 2006 [149]	Cross-sectional hospital-based survey of healthy postmenopausal women. (N=950)	BMD	Individuals with low normal TSH levels (0.5-1.1 mU/l) had a 2.2-fold increased risk of osteoporosis than those with high normal TSH levels (2.8-5.0 mU/l).
Morris USA 2007 [150]	Cross-sectional study of healthy post-menopausal women from the National Health and Nutrition Examination Survey (NHANES) (N=581)	BMD	Lower levels of TSH were associated with lower levels of BMD. Individuals with a TSH level within the reference range, but below the median level (1.8 mU/l) had increased odds of osteoporosis.
Grimnes Norway 2008 [153]	5th Tromsø study (N=1,961 M:F 1:1.03)	BMD	Individuals with serum TSH below the 2.5 percentile had significantly lower BMD at the ultra-distal (women) and distal (both sexes) forearm than those with serum TSH in the normal range. However, within the normal range of serum TSH, serum TSH was not associated with BMD. This study had a relatively high proportion of males compared to similar studies in this area; which may explain the lack of association between TSH and BMD.
Murphy 2010 [156]	Osteoporosis and ultrasound study (OPUS) a 6-year prospective population cohort	BMD	Within the reference range, FT3 (β =-0.087 p=0.005) and FT4 (β =-0.091; p=0.004) were negatively associated

	study of healthy euthyroid postmenopausal women (N=1,278)		with BMD at the hip. Per unit increase, even after adjusting for age, BMI and BMD, the risk of non-vertebral fracture was increased by 33% in individuals with higher FT3 (p=0.006) and 20% in individuals with higher FT4 (p=0.002). In contrast, higher levels of TSH were protective and each unit increase in TSH reduced the risk of non-vertebral fracture by 35% (p=0.028). FT3 was associated with key falls risk factors being positively associated with grip strength p=<0.001) and balance (p=<0.001).
Kim Korea 2010 [151]	Cross-sectional study of healthy euthyroid men (N=1,478)	BMD	The odds of having osteopenia and osteoporosis were increased in subjects with low-normal TSH (0.4-1.2 mU/l), when compared to high-normal TSH (3.1-5.0 mU/l), after adjustment for confounding (OR = 1.45, 95%CI = 1.02 - 2.10). e.
Mazziotti Italy 2010 [152]	Cross-sectional study of post-menopausal women with normal thyroid function but low BMD (N=130)	Vertebral fractures	Vertebral fractures were found to be significantly (*p* = 0.004) more prevalent in first tertile (56.8%) of TSH values as compared with the second (23.3%) and third tertiles (32.6%). Individuals with lower serum TSH also had increased odds of vertebral fractures (OR=2.8, 95%CI 1.20–6.79) even after

			correction for age, BMD, BMI and serum FT4.
Roef Belgium 2011 [155]	Cross-sectional study of 677 healthy male siblings aged 25-45 years	BMD	There was lower hip BMD with increasing FT3 levels within the reference range but no association was seen with FT4 or TSH. FT3 was also negatively associated with lumbar spine BMD (β =-0.10, p=0.008) but no association was identified with FT4 or TSH

BMD = bone mineral density, BMI = body mass index, OR= odds ratio TSH = thyroid stimulating hormone

3.3.4 Overview

A summary of adverse outcomes by TSH level is shown in **Table 10**. A fixed-effects meta-analysis of the odds ratios of adverse health outcomes for higher TSH levels within the reference-range compared to lower levels of TSH is shown in **Figure 6**. There was very strong evidence in the fixed-effects meta-analysis that individuals with TSH levels in the upper part of the reference-range had increased odds of adverse cardiovascular outcomes OR=1.21 (95%CI 1.15-1.27) p=7.99 $x10^{-15}$ and adverse metabolic outcomes OR=1.37 (95%CI 1.27-1.48) p=5.99 $x10^{-15}$ but lower odds of adverse bone outcomes OR=0.55 (95%CI 0.41-0.72) p=1.93 $x10^{-05}$ compared to individuals with TSH levels in the lower part of the reference-range. Similar associations were observed in the random-effects model **(Figure 7**). Overall the models produced similar results, however the random-effects is the more desirable, as the fixed effects assumes similar effects for each component studied. For instance in the various components of cardiovascular outcomes (hypertension, cholesterol and mortality) it is unlikely that variation in TSH has similar effects for each component.

Table 10 Summary of the odds of adverse outcomes for higher TSH levels in the top third of the reference-range against TSH levels in the lower third of the reference-range

Report	Outcome	Odds ratio	95%CI	P value
Cardiovascular Outcomes				
Asvold 2007	Hypertension (F)	1.23	(1.04, 1.46)	0.02
Asvold 2007	Hypertension (M)	1.98	(1.56, 2.53)	<0.001
Itterman 2012	Hypertension (Children)	1.12	(1.00, 1.25)	0.05
Itterman 2012	Hypertension (Adults)	1.19	(1.12, 1.26)	<0.001
Asvold 2008	CVS mortality (F)	1.69	(1.14, 2.52)	0.01
Asvold 2008	CVS mortality (M)	1.20	(0.76, 1.88)	0.43
Metabolic Outcomes				
Kim 2011	Metabolic Syndrome	1.92	(1.24, 2.98)	0.004
Ruhla 2010	Metabolic Syndrome	1.70	(1.11, 2.60)	0.01
Asvold 2009	Obesity (F)	1.30	(1.16, 1.46)	<0.001
Asvold 2009	Obesity (M)	1.53	(1.26, 1.84)	<0.001
Asvold 2011	CKD	1.31	(1.13, 1.52)	<0.001
Bone Outcomes				
Morris 2007	Osteoporosin (F)	0.29	(0.11, 0.77)	0.01
Kim 2006	Osteoporosis (F)	0.45	(0.25, 0.83)	0.01
Kim 2010	Osteoporosis (M)	0.69	(0.48, 0.98)	0.04
Mazzioti	Vertebral fracture (F)	0.35	(0.15, 0.83)	0.02

OR = Odds ratio
95%CI = 95% confidence interval
F= Female
M = Male
CVS = Cardiovascular
CKD = Chronic Kidney disease

Figure 6 The odds of adverse outcomes for higher TSH levels within the reference range compared to lower levels of TSH within the reference range with fixed-effects meta-analysis.

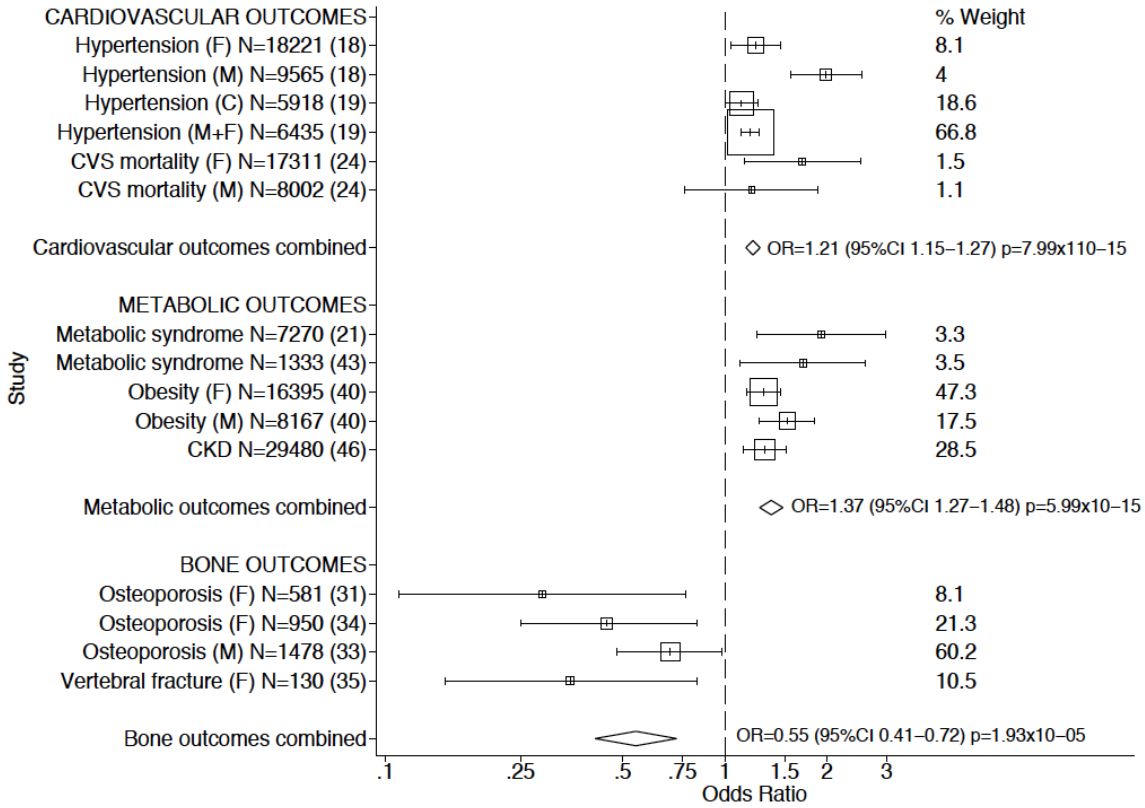
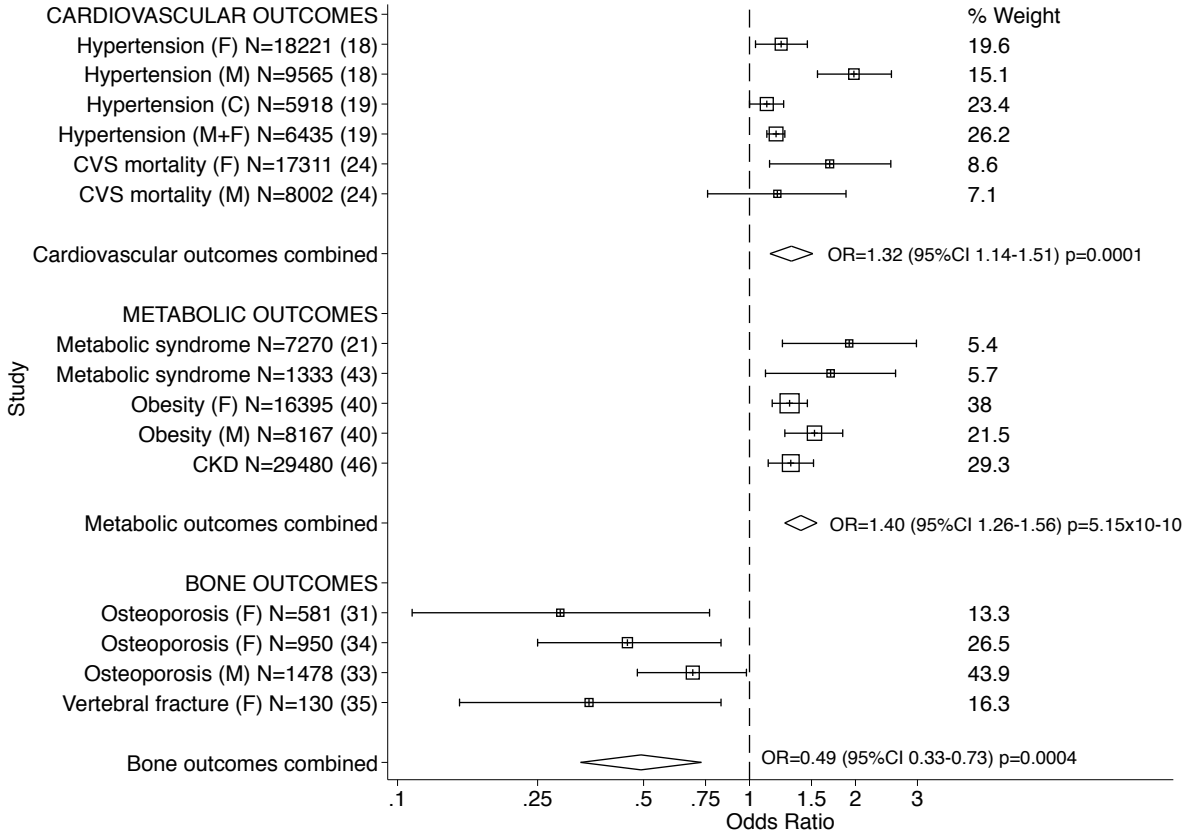


Figure 7 The odds of adverse outcomes for higher TSH levels within the reference range compared to lower levels of TSH within the reference range with random-effects meta-analysis.



Tau^2 for random effects.Cardiovascular outcomes p=0.02 Metabolic outcomes p=0.04 Bone outcomes p=0.06

3.3.5 The effect of variation in thyroid status within the population reference range on neuropsychological outcomes

Data are summarized in **Table 11**. Whilst thyroid dysfunction results in impaired CNS development [176] and possibly mood [82], the impact of variation within the population reference-range is less clear. Analysis in HUNT identified that there may be interaction by sex on the association between TSH and mood [157]. In this study [157] there was an inverse association between serum TSH and depression in males, but no evidence of association in females. In females on levothyroxine, TSH was **positively** associated with both depression and anxiety. Analysis of neuropsychological outcomes in cohorts of children [177, 178] and older individuals have been inconclusive [29, 156, 179-181] most likely due to lack of power as these cohorts have been smaller and are prone to type-2 error or type-1 error and subsequent publication bias.

A meta-analysis identified a positive association between depression and FT4 within the reference-range OR=1.12, (95%CI 1.02-1.22) p=0.01 [156]. This is in keeping with the observed inverse association between TSH and depression in males in the HUNT study [157], but in contrast to traditional thinking that higher levels of TSH are associated with increased levels of depression. Studies of selective cohorts of individuals with depression are inconsistent; individuals with serum TSH concentrations in the upper 25th percentile of the normal range were more likely to have more episodes of major depression, longer duration of depression and a higher number of suicide attempts than patients who had serum TSH concentrations

below the upper 25th percentile of the reference-range [182]. However in another cohort of individuals with depression, those with a high-normal TSH (≥2.5mU/l) had lower depression as measured by Hamilton Depression Rating scores, fewer anxiety symptoms and less suicidal ideation than those with low-normal TSH (<2.5mU/l) [183].These data are not from the general population and observed associations may instead be due to selection bias, medication effects and reverse causation through the effects of major depression on the hypothalamic-pituitary-thyroid axis.

Table 11 The effect of variation in thyroid function within the population reference range on neurological/psychological outcomes

Report	Study Type	Outcome	Comments
Wahlin Sweden 1998 [181]	Cross-sectional analysis of a non demented elderly population cohort (N=200)	Memory skills	TSH was positively related to episodic memory performance. No association was observed with FT4.
Pop Belgium 1999 [176]	Cohort of children, born to women with without thyroid disease (N=220 M:F 1:2.35)	Childhood development	Children of mothers with low thyroid hormone (below the 10th centile) at 12 weeks' gestation had lower scores on the Bayley Psychomotor Developmental Index scale at 10 months of age.
Berlin France 1999 [182]	In-patients with DSM III-R criteria for major depression. (N=94 M:F 1:2.36)	Depression status and duration	Individuals who had serum TSH concentrations greater than the upper 25th percentile of the normal range were more likely to have higher number of episodes of major depression
Van Boxtel Netherlands 2004 [180]	Random sample of the Maastrict Aging Study (N=120 M:F 1:1)	Memory function	There was a modest negative association between TSH and memory function.
Gussekloo Netherlands [29]	Prospective cohort study of older individuals aged over 85. (N=559 M:F 1:2)	Depressive symptoms and cognition	TSH and FT4 were not associated with disability in daily life, depressive symptoms, and cognitive impairment.
Eskelinen Finland 2007	Cross-sectional study of	Quality of life scores MMSE	There were no associations between TSH levels

[179]	indiviuals without thyroid disease (N = 1086 M:F 1:1.16)		and self-rated health or life satisfaction,
Alvarez-Pedrerol Spain 2007 [177]	Cross-sectional study from 2 small population cohorts in Menorca and Ribera d'Ebre (N=342 M:F 1:1.04)	Attention deficit and hyperactivity	Children in the highest quartile for TSH, had greater odds of having more than six attention deficit symptoms and 1–5 hyperactivity or impulsivity symptoms In contrast, high FT4 levels were associated with decreased odds of having 1-5 attention deficit symptoms.
Joffe USA 2008 [183]	Cross-sectional study of individuals with major depression (N=166)	Depression	Individuals with higher thyroid function were significantly more depressed, as measured by Hamilton Depression Rating Scale scores, and had more anxiety symptoms and suicidal tendencies than those with lower thyroid function.
Panicker Norway 2009 [157]	Cross-sectional analysis of a subgroup of individuals in the HUNT 2 study with serum TSH and mood scores. Stratified by those on (N=1,265 all female) and not on levothyroxine	Depression and anxiety	There were clear differences in the relationship between TSH and mood in males and females and also in those on levothyroxine.

	(N=27,013 M:F 1:1.90)		
Williams UK 2009 [156]	Prospective cohort study of middle aged men (45-59 years) (N=2,269 all male	Psychiatric morbidity (GHQ-30) over a mean of 12.3 years follow-up.	There was a positive association between total T(4) and chronic psychiatric morbidity
Hoshiko USA 2011 [178]	Case-control study of children with autistic spectrum disorder. (N cases = 554 with regional controls N = 784)	Autistic spectrum disorder	Infants with very low FT4 (lower than the third percentile) may have a higher risk of autism.

OR = odds ratio, TSH = thyroid stimulating hormone

3.4 DISCUSSION

We have highlighted that variation in thyroid hormone levels within the population reference-range is associated with a wide range of adverse health outcomes. Higher TSH levels are associated with worse cardiovascular risk factors, metabolic parameters and pregnancy outcomes, whereas lower TSH levels are associated with reduced BMD and increased risk of osteoporosis and fracture. The evidence-base for our findings was generally good for cardiovascular, metabolic, bone and pregnancy outcomes, being derived from large population cohorts; however high-quality data remains lacking for neurological outcomes and most psychological outcome studies were under-powered.

A key aim in studying the relationships between thyroid function within the general population and health outcomes was to take advantage of large study populations without selection bias to inform the debate on thresholds for treating subclinical thyroid disease. Hence, it might be expected that effects attributable to variation in thyroid function across the reference-range would be similar if not greater in subjects with thyroid function outside this range. The data collated in this report suggests that, at least at the population level, treatment of subclinical thyroid disease could potentially improve health outcomes. However, important limitations need to be taken into account in extrapolating data from the reference-range to assess risk of adverse outcomes for individuals with subclinical thyroid disease. Most studies in this report have been in individuals of white European ancestry which limits

generalizability. The majority of studied associations were also with TSH only, data are still lacking on the phenotypic consequences of variation in FT3 and FT4. Furthermore a substantial proportion of identified associations were from cross-sectional analyses, which are prone to unmeasured/residual confounding and reverse causation. For example, cigarette smoking is associated with reduced TSH concentrations; [184] this makes it difficult to accurately assess the effect of thyroid dysfunction on association studies with smoking-dependent outcomes, such as cardiovascular disease [141].

There may also be publication bias as negative studies assessing the effect of variation of thyroid function in the population reference-range on outcomes may be hard to publish.

When considering whether to treat subclinical hypothyroidism, there also needs to be careful consideration of the complexities of thyroid hormone replacement. For instance the population reference-range by far exceeds the variation of the intra-individual set point [71], and although levothyroxine treatment will restore an individual's TSH levels to within the 'normal population range', this may be outside their genetically determined set-point [185]. It is also unclear whether treatment with levothyroxine in individuals with subclinical hypothyroidism will normalize the odds of developing adverse outcomes, for instance treating individuals with levothyroxine will substantially reduce their T_3:T_4 ratio [186] and the long-term consequences of this in patients with subclinical

hypothyroidism are currently unclear. The pituitary response as measured by changes in TSH may not fully reflect the thyroid status in other key organs; for example common variation in *DIO2* has been shown to influence mood and response to combination T_3/T_4 therapy [187] and osteoarthritis risk [188], but has no effect on serum thyroid hormone levels [187]. Hence it is possible, that improving outcomes for cardiovascular disease, may at the same time increase the risk of osteoporosis in the same individual and the optimal TSH (or indeed the premorbid TSH) may be difficult to determine. Furthermore with current practice 40-48% of hypothyroid patients on levothyroxine do not achieve target TSH values [42, 189] with many individuals over-treated. Taken together, this data provides a strong rationale for the treatment of subclinical thyroid disease, but large, carefully designed, long-term, randomized clinical trials will be needed to determine the true balance of benefits and risks, and optimal thresholds for intervention.

The continuum of effects across the reference-range of thyroid function, suggest that it might be more appropriate to consider thyroid hormone levels as "risk factors" for disease (similar to blood pressure or cholesterol in cardiovascular disease), rather than consider a particular level to be "normal" or "abnormal". In this way of thinking, the net benefit of intervention at a particular TSH level, can be related to an individual's comorbidities. For example, more net benefit might be obtained in initiating levothyroxine therapy for subclinical hypothyroidism in an adult with multiple cardiovascular risk factors than

in one with osteoporosis. This approach might then suggest that younger adults with cardiovascular risk factors, should be screened for thyroid disease, as this will increase the likelihood of identifying patients with subclinical disease who might benefit most from intervention [190, 191].

Considering thyroid hormone levels as continuously distributed risk factors for different health outcomes may also help inform the debate on the upper limit of "normal" TSH The National Academy of Clinical Biochemists highlighted that 95% of individuals without evidence of thyroid disease or autoantibodies had TSH concentrations below 2.5mU/l [192] leading for calls to lower it to this level [90]. However it has been argued that lowering the upper TSH limit is unnecessary as treating individuals with high-normal TSH is unwarranted and routine levothyroxine treatment is not currently recommended for subclinical hypothyroidism [91]. Identifying TSH levels at which net benefit for intervention can be obtained by treatment in different patient groups by prospective studies may be a more relevant goal.

Although no large prospective intervention studies have been performed in subclinical hypothyroidism, there have been cohort studies in this area. A large individual patient data meta-analysis (N=55,287) from 11 population cohorts [193] identified that the impact of subclinical hypothyroidism on coronary heart disease event only became apparent between a TSH level of 7.0-9.9mU/l HR=1.17 (95%CI 0.96-1.43), with no clear effect observed for TSH levels between 4.50–6.99mU/l HR=1.00

(95%CI 0.86-1.18). However levothyroxine treatment at TSH levels lower than 7.00mU/l, especially in younger individuals, may still be beneficial; analysis from the UK General Practice Research Database identified in individuals under the age of 70 levothyroxine treatment at TSH levels between 5-10mU/l reduced the future risk of IHD events HR=0.61 (95%CI 0.39-0.95) [191]. This is in keeping with our observed differences in the odds of adverse cardiovascular outcomes within even the population range.

In summary, the data in this chapter has identified that variation in thyroid hormone parameters within the population reference-range resulted in increased odds of adverse outcomes, but this should not be used as justification for treating at risk individuals with thyroid hormone parameters within the reference-range (pregnancy aside). In particular, data does not support levothyroxine treatment with TSH levels within the reference-range for low mood. The potential benefits of treating individuals within the normal population range would only be modest and over-replacement with levothyroxine is associated with osteoporosis and atrial fibrillation [160].

This analysis has highlighted that modest variation in thyroid hormone levels are associated with increased odds of developing a wide range of adverse health outcomes. Prospective clinical trials in subclinical hypothyroidism, which recognize the complexities of thyroid hormone replacement, are therefore urgently required. In particular, adequately

powered prospective, randomized, controlled, double-blinded long-term interventional trials will be required to fully identify the benefits and risks of treatment as well as determining appropriate TSH thresholds for intervention in different patient groups.

Data here has also indicated that over treating borderline thyroid function with levothyroxine, may simply change adverse cardiovascular and metabolic risk factors for adverse bone outcomes.

Chapter 4 The relationship between thyroid function and body composition in children

As I observed earlier in this thesis variation in TSH levels in adults even across the population reference-range is associated with substantial differences in body mass index and body composition **[143, 145, 146]**. The relationship between thyroid status and body composition is less well understood in children and this is the focus of the chapter. However given the importance of thyroid status on growth and development, an exploration of the longitudinal stability of TSH and thyroid hormone levels is also necessary.

This Chapter has been separated into 3 parts; **4A** where I explore the longitudinal stability of TSH and thyroid hormone levels in children as assessments of body composition were undertaken at in early and late puberty. In part **4B** I will explore the relationship between thyroid status in children and body composition and in part **4C** I will use instrumental variable analyses to help assess the relationship between FT3 levels and body composition as in part **4B** I observed an unexpected relationship between FT3 and fat mass.

4A.1 INTRODUCTION

There is limited data on thyroid hormone reference ranges in children, particularly with regard to FT3 levels. Whilst it is well established in adults that there is narrow intra-individual variation in thyroid hormone

parameters compared to inter-individual variation [71] increased variance in thyroid hormone levels has been observed throughout childhood and adult reference intervals may not be universally applicable to children [74, 75, 194]. Previous cross-sectional studies from convenience samples have indicated that FT3 substantially falls and FT4 rises from age 4 [55, 73, 195] but there have been no longitudinal studies to confirm these observations.

In this original analysis, I therefore described age and sex reference-ranges in 4,442 children at age 7 and 1,253 children at age 15 (884 children had thyroid function measured at both time points). I also explored the longitudinal variability of TSH and thyroid hormone levels using linear mixed models by sex and pubertal status and also assessed the relationship between TSH and thyroid hormone at different time-points over childhood.

4A.2 METHODS

4A.2.1 Study participants, laboratory and phenotypic measures

The ALSPAC cohort has been described in detail in Chapter 2. Serum TSH, FT_3 and FT_4 were measured at age 7 (median age 89 months) by chemiluminescent emission using a photomultiplier on cobas® e601 (Roche Diagnostics, Mannheim Germany) in 4,442 children. Thyroid function at age 15 was also performed in 1,253 children (median age 184 months) using the same method and was available at both age 7 and 15 in 884 children. Reference-ranges for adults are TSH, 0.27-4.2 mU/liter,

FT3 3.9-6.7pmol/liter, FT4 12-22 pmol/liter. Samples were collected from 1997 onwards with analysis performed in 2010-2011; it has been previously demonstrated that TSH and FT_4 can be analyzed reliably in samples stored for up to 23 years [196].

Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain Ltd., Crymych, UK). Pubertal status was self-assessed using a Tanner stage questionnaire at age 13.5 years (pubic hair domain) range 13.1 to 14.4 years. Pubertal status used in this analysis was stage P1 - prepubertal, lanugo may be present in genital area but it is fine and downy. Stage P2 - sparse growth of pubic hair in the midline, mainly at the base of the penis or along the labia majora. Stage P3 - more hair grows so that it is visible from several feet, along with coarsening and increased pigmentation in some people.

One possible confounder for TSH and thyroid hormone levels is iodine intake. Iodine rich foods are white fish and dairy products which may have a social class bias therefore additional adjustments were made for home circumstances including age of mother at birth of child, parity of mother at birth of child (1/1-4/more than 4) maternal smoking during pregnancy (none/some), educational status of mother (low=no qualifications, certificate of secondary education, or vocational/medium=O level/high=A level or degree), Housing status (owned or mortgaged/privately rented/council rented), Family adversity index (see **Appendix 1**), Home score (1-4/4-8/9-12 -see **Appendix 1**).

4A.2.2 Statistical Analysis

Before any analyses were conducted each variable was examined carefully using histogram generation and cross-tabulation to identify any errors, inconsistencies or grossly abnormal results. For example, males and females were examined to ensure they had appropriate corresponding pubertal measurements. To ensure the appropriateness of using linear regression histograms were generated to clarify whether the assumptions of normality of distribution were valid.

Categorical variables were checked for impossible values and any identified were recoded as missing. Implausible TSH and thyroid hormone levels (> 4 SD from the mean for the sex and age-specific category) were considered as outliers and were recoded to missing. TSH was $\log_e$ transformed to an approximately normal distribution. Descriptive statistics are presented as geometric means, standard deviations (SD), median and 95th centiles.

A linear mixed model with random intercepts and random slopes was used to assess the trends of TSH and thyroid hormone parameters over childhood [197]. An unstructured variance-covariance matrix was assumed. We analyzed the baseline values at age 7, the variability at baseline, the longitudinal trend (slope) between age 7 and 15 and the variability in the slope. Analyses were performed with sex interactions

and sex X puberty interactions. Model simplification was undertaken using likelihood ratio tests.

We then explored the relationship between TSH and thyroid hormone levels at ages 7 and 15. Here thyroid function was standardized and therefore results are presented as per SD change in the outcome. Analyses were initially performed adjusted for age at thyroid measurements and sex (model 1). Three further models controlling for key potential confounders were undertaken; model 2 also adjusted for thyroid hormone parameters, model 3 also adjusted for measures of social class and early life environment including home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score. Likelihood ratio tests were used to identify if there was any evidence of interaction by sex on the relationship between thyroid hormone parameters and TSH.

4A.2.3 Funding of thyroid function performed in ALSPAC

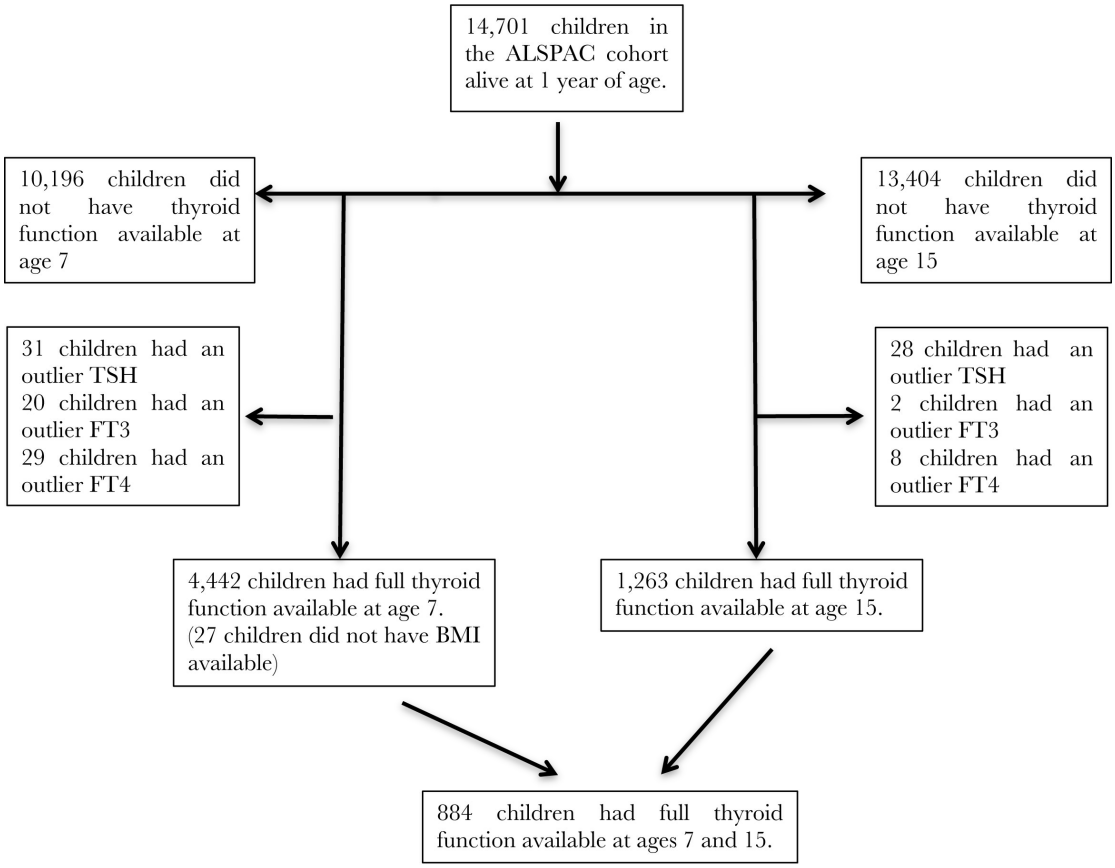
Thyroid function testing in ALSPAC was undertaken using 2 grants obtained by myself and Professor Dayan. These included a BUPA health research grant of £45,000 and an Above and Beyond Grant of £10,000. The majority of the grants were spent on performing thyroid function at age 7, however we had some funds to also perform thyroid function at age 15. Age 7 was prioritized over age 15 as data in younger children on the role of thyroid function was more limited and this was done to maximize the benefits of the grant.

4A.3 RESULTS

4A.3.1 Study population and baseline characteristics

The derivation of study participant numbers is shown in **Figure 8.**

Figure 8 Study participants



Children in our study dataset were more likely to have several higher markers of affluence and fewer early life events than the remainder of the ALSPAC cohort (**Table 12**). This may influence generalizability, although no clear relationship were seen with thyroid status and social class in analyses.

Table 12 Comparison of the study cohort to the remainder of the ALSPAC cohort

Variable	Study Cohort	Remaining ALSPAC cohort†	*p value
Child Sex (% Male)	52.3	50.9	0.14
Family Adversity Index Mean (SD)	4.67 (4.42)	3.88 (3.94)	<0.001
Home Score			0.003
0-4 (%)	3.39	4.64	
5-8 (%)	24.0	24.0	
9-12 (%)	72.6	72.5	
Housing Status			<0.001
Owned/mortgaged (%)	82.9	69.1	
Privately rented (%)	13.3	25.1	
Council rented/other (%)	3.8	5.8	
Maternal age at birth of child (years) Mean (SD)	29.1 (4.55)	27.5 (5.06)	<0.001
Maternal highest educational status			<0.001
Low (%)	20.9	34.4	
Middle (%)	35.1	34.3	
High (%)	44.0	31.3	
Maternal smoking in pregnancy			<0.001
None (%)	82.1	72.2	
Some (%)	17.9	27.8	
Parity			<0.001
0 - 1 (%)	81.1	79.2	
2 – 4 (%)	18.7	20.2	
> 5 (%)	0.2	0.7	

*Calculated using the Wald test
†Remaining ALSPAC cohort defined as women who enrolled in the core ALSPAC sample with children surviving to 1 year (N= 14,701) p = strength of evidence against the null hypothesis of no difference in characteristics between study population and remainder of the ALSPAC cohort

4A.3.2 Serum thyroid hormone levels in children at ages 7 and 15

At age 7 years, the mean and 95% reference range values for TSH, FT_3 and FT_4 were 2.26 (0.93 - 4.48) mU/l 6.29 (5.13 - 7.59) pmol/l and 15.7 (12.7 – 19.3) pmol/l respectively (**Table 13**). 23.2% of children at age 7 years had a FT_3 above the adult reference range, with only 3.65% of

children having a TSH and 0.2% of children having FT_4 values above the adult reference-range (**Table 13 Figure 9**). At age 15 years, the mean and 95% reference range values for TSH, FT_3 and FT_4 were 2.43 (0.91 – 5.05) mU/l 5.83 (4.45 - 7.35) pmol/l and 15.5 (11.9 – 20.3) pmol/l respectively (**Figure 10**), with a marked reduction in children having FT_3 above the adult reference-range to 12.2% (**Table 13**).

Table 13 Reference-range for thyroid hormone parameters age 7 and age 15

	Age (years)	All					Males					Females				
		N	Mean	(2.5-97.5%)	% above ARR	% below ARR	N	Mean	(2.5-97.5%)	% above ARR	% below ARR	N	Mean	(2.5-97.5%)	% above ARR	% below ARR
TSH (mU/l)	7	4,442	2.26	0.93 - 4.48	3.65	0	2,323	2.32	0.97 - 4.50	3.57	0	2,119	2.20	0.88 – 4.45	3.73	0
FT3 (pmol/l)		4,442	6.29	5.13 – 7.59	23.2	0.09	2,323	6.23	5.07 – 7.56	19.8	0.17	2,119	6.35	5.16- 7.59	26.9	0
FT4 (pmol/l)		4,422	15.7	12.7 – 19.3	0.20	0.70	2,323	15.6	12.7 – 19.0	0.17	0.73	2,119	15.9	12.9 – 19.6	0.24	0.66
TSH (mU/l)	15	1,263	2.43	0.91 – 5.05	6.33	0	644	2.51	0.91- 5.17	7.92	0	619	2.34	0.87 – 5.00	4.68	0
FT3 (pmol/l)		1,263	5.83	4.45 – 7.35	12.2	0.55	644	6.16	4.84 – 7.6	20.7	0	619	5.48	4.23 – 6.91	3.39	1.13
FT4 (pmol/l)		1,263	15.5	11.9 – 20.3	0.79	2.69	644	15.5	11.8 – 20.2	0.62	2.95	619	15.5	12.0 – 20.6	0.97	2.42

N=Number
ARR= Adult reference range
TSH = Thyroid stimulating hormone
FT3 = Free tri-iodothyronine
FT4 = Free thyroxine

Figure 9 Histograms of TSH, FT3 and FT4 levels at age 7

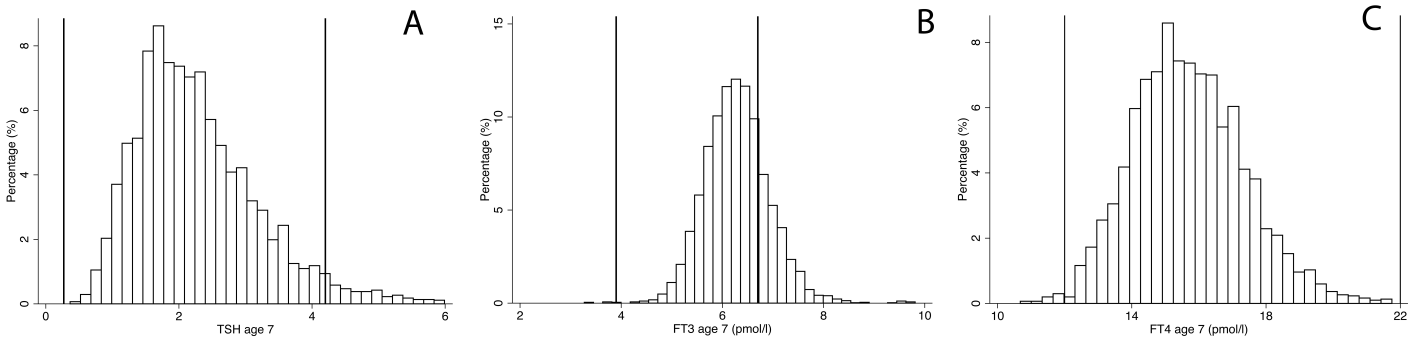
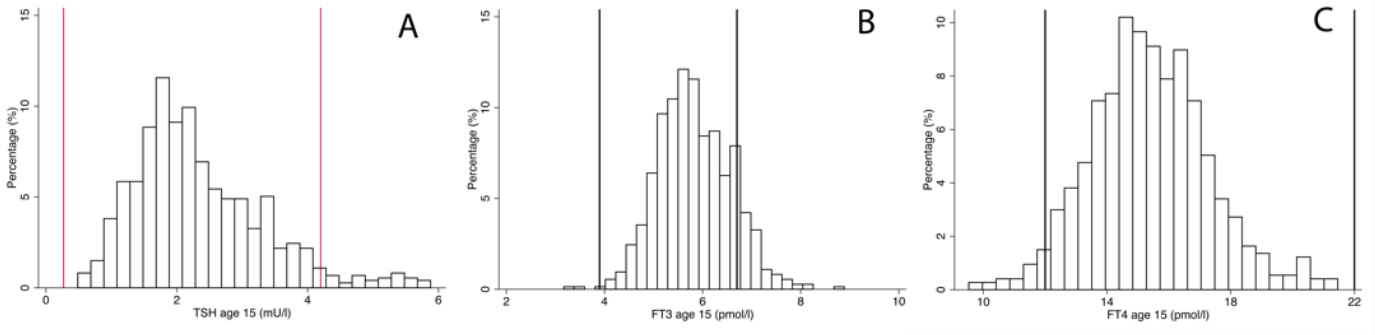


Figure 10 Histograms of TSH, FT3 and FT4 levels at age 15



Vertical bars represent the limits of reference range for adults (TSH, 0.27-4.2 mU/l, FT3 3.9-6.7pmol/l, FT4 12-22 pmol/l)

4A.3.3 Linear mixed models analysis in children with thyroid function at age 7 and age 15

TSH levels rose between ages 7 and 15 years whereas both FT_3 and FT_4 levels fell. Strong negative correlations were observed in the models for TSH, FT_3 and FT_4 indicating that those with higher levels at age 7 years were more likely to have greater reductions, and those with lower levels at age 7 were likely to have small reductions by age 15, i.e. a convergence of biomarkers (**Table 14**). Every 2 years between ages 7 and 15 years, TSH levels increased by 0.03 mU/l (95%CI 0.02, 0.05) p <0.001. Boys had a higher baseline TSH than girls at age 7 years of 0.11mU/l (95%CI 0.06, 0.17) p <0.001. There was no difference in mean gain between boys and girls between ages 7 and 15 years β=0.0001 (95%CI -0.001, 0.001) p=0.83 and no difference in variability at baseline -0.04 (95%CI -0.10, 0.03) p=0.29 or in the variability of the slope β= $5.73x10^{-06}$ (95%CI -0.0002, 0.0003) p=0.65 (**Tables 15 and 16**).

For FT_3 every 2 years between the ages of 7 and 15 years, FT_3 levels fell 0.12 pmol/l (95%CI -0.13, -0.10). Girls had a higher baseline FT_3 level than boys 0.13pmol/l (95%CI 0.09, 0.17) p <0.001. However boys had a more substantial gain in FT_3 than girls B=0.008 (95%CI 0.007, 0.009) p<0.001. There was no substantial difference by sex in variability at baseline B=0.02 (95%CI -0.01, 0.05) p=0.29, or in variability in slope β = $7.85x10^{-06}$ (95%CI $-5.18x10^{-06}$, $2.01x10^{-05}$) p=0.24 (**Tables 14-16**). Every 2 years FT_4 levels fell 0.04 pmol/l (95%CI -0.07, -0.01) p=0.005. Girls had a higher baseline FT_4 level than boys of 0.38pmol/l (95%CI 0.28, 0.48) p<0.001, however boys had a higher mean gain β =0.004 (95%CI 0.002, 0.006)

p=0.001. As a result boys had a higher FT_3 than girls by age 15. Girls also had more variability at baseline at age 7 years β =0.38 (95%CI 0.14, 0.62) p=0.002 although there was no difference in variability in slope $\beta=4.47x10^{-05}$ (95%CI $5.12x10^{-07}$, 0.001) p=0.33 (**Tables 15 and 16**).

Table 14 Overall linear mixed models for TSH FT3 and FT4

Parameter				β	95% CI		P-Value
TSH (mU/l)	All	Main effects	Age 7 years	2.27	2.24,	2.32	
			Slope	0.0013	0.0007,	0.002	<0.001
		Variability	SD@ Age 7 years	1.62	1.56	1.67	
			SD Slope	0.14	0.13,	0.14	
			Correlation(int,slope)	-0.87	-0.89,	-0.86	
FT3 (pmol/l)	All	Main effects	Age 7 years	6.29	6.27,	6.31	
			Slope	-0.005	-0.005,	-0.004	
		Variability	SD@ Age 7 years	1.28	1.24,	1.32	<0.001
			SDSlope	0.12	0.11,	0.12	
			Correlation(int,slope)	-0.92	-0.93,	-0.91	
FT4 (pmol/l)	All	Main effects	Age 7 years	15.7	15.7,	15.8	
			Slope	-0.002	-0.03,	-0.0005	0.005
		Variability	SD@ Age 7	3.03	2.92	3.14	
			SDSlope	0.27	0.26,	0.28	
			Correlation(int,slope)	-0.86	-0.88,	-0.84	

SD = Standard Deviation
95%CI = 95% Confidence interval

Table 15 Linear mixed models for TSH FT3 and FT4 in boys

Parameter				β	95% CI		P-Value
TSH (mU/l)	Boys	Main effects	Age 7	2.32	2.28	2.36	<0.001
			Slope	0.001	0.0005	0.002	0.002
		Variability	SD@ Age 7	0.9	0.87	0.92	
			SDSlope	0.01	0.01	0.01	
			Correlation(int,slope)	-0.46	-0.52	-0.39	
FT3 (pmol/l)	Boys	Main effects	Age 7	6.23	6.2	6.25	<0.001
			Slope	-0.0005	-0.001	0.00009	0.09
		Variability	SD@ Age 7	0.63	0.61	0.64	
			SDSlope	0.009	0.008	0.009	
			Correlation(int,slope)	-0.58	-0.63	-0.53	
FT4 (pmol/l)	Boys	Main effects	Age 7	15.5	15.4	15.6	<0.001
			Slope	0.0003	-0.001	0.002	0.72
		Variability	SD@ Age 7	1.63	1.58	1.68	
			SDSlope	0.022	0.021	0.023	
			Correlation(int,slope)	-0.41	-0.48	-0.34	

SD = Standard Deviation
95%CI = 95% Confidence interval

Table 16 Linear mixed models for TSH FT3 and FT4 in girls

Parameter				β	95% CI		P-Value
TSH (mU/l)	Girls	Main effects	Age 7	2.21	2.17	2.24	<0.001
			Slope	0.001	0.0004	0.002	<0.001
		Variability	SD@ Age 7	0.92	0.89	0.95	
			SDSlope	0.01	0.01	0.01	
			Correlation(int,slope)	-0.52	-0.58	-0.46	
FT3 (pmol/l)	Girls	Main effects	Age 7	6.36	6.33	6.38	<0.001
			Slope	-0.009	-0.01	-0.08	<0.001
		Variability	SD@ Age 7	0.61	0.59	0.63	
			SDSlope	0.009	0.008	0.009	
			Correlation(int,slope)	-0.61	-0.66	-0.56	
FT4 (pmol/l)	Girls	Main effects	Age 7	15.9	15.8	16	<0.001
			Slope	-0.004	-0.006	-0.002	<0.001
		Variability	SD@ Age 7	1.74	1.69	1.8	
			SDSlope	0.022	0.21	0.22	
			Correlation(int,slope)	-0.42	-0.49	-0.35	

SD = Standard Deviation
95%CI = 95% Confidence interval

4A.3.4 Relationship between pubertal status at age 13 and TSH and thyroid hormone parameters at aged 7 and 15

2,702 children also had pubertal status self-assessed at age 13 years as well as having thyroid function measured. Pubertal status at age 13 years was not associated with TSH levels at age 7 in boys (p=0.89) or girls (p=0.31). No detectable difference in TSH slope by pubertal status was observed in boys (p=0.82) or girls (p=0.82). Pubertal status at age 13 years was not also associated with FT_4 levels at age 7 years in boys (p=0.32) or girls (p=0.52). However, FT_3 levels at age 7 years were higher in boys (p=0.0001) and girls (p=0.04) with more advanced puberty at age 13 years (**Tables 17 and 18**). More advanced pubertal status at age 13 years was however associated with a negative FT_3 slope unlike early pubertal status which had a positive FT_3 slope in both boys and girls (p=<0.001). This suggests that FT3 levels may peak in the very early stages of puberty as these changes appear to occur even before age 7. The negative slope indicates that FT3 falls throughout puberty after this peak. There was weak evidence of any difference in the variability of baseline values or gradients of slopes by pubertal status in either boys or girls for either FT_3 or FT_4.

Table 17 Linear mixed models for TSH FT3 and FT4 by pubertal status in boys (Tanner pubic hair domain) at age 13 years

				P1				P2				P3			
Parameter				β	95% CI		P-Value	β	95% CI		P-Value	β	95% CI		P-Value
TSH	Boys	Main effects	Age 7	2.37	2.28	2.46	<0.001	2.4	2.3	2.51	<0.001	2.38	2.29	2.46	<0.001
			Slope	0.002	0	0.004	0.05	0.001	-0.001	0.003	0.19	0.001	-0.001	0.002	0.26
		Variability	SD@ Age 7	0.92	0.85	0.98		0.94	0.86	1.01		0.94	0.88	1.01	
			SDSlope	0.01	0.01	0.01		0.01	0.01	0.01		0.01	0.01	0.01	
			Correlation(int,slope)	-0.47	-0.59	-0.34		-0.41	-0.57	-0.26		-0.46	-0.58	-0.33	
T3	Boys	Main effects	Age 7	6.14	6.08	6.2	<0.001	6.18	6.11	6.25	<0.001	6.32	6.26	6.38	<0.001
			Slope	0.002	0.0003	0.003	0.01	0	-0.001	0.001	0.99	-0.003	-0.004	-0.002	<0.001
		Variability	SD@ Age 7	0.63	0.59	0.68		0.59	0.54	0.64		0.63	0.58	0.67	
			SDSlope	0.008	0.007	0.009		0.01	0.008	0.01		0.009	0.008	0.01	
			Correlation(int,slope)	-0.53	-0.64	-0.41		-0.66	-0.77	-0.56		-0.62	-0.71	-0.52	
T4	Boys	Main effects	Age 7	15.6	15.4	15.7	<0.001	15.6	15.5	15.8	<0.001	15.5	15.3	15.6	<0.001
			Slope	-0.004	-0.007	-0.001	0.02	-0.004	-0.008	-0.001	0.02	0.005	0.002	0.009	0.001
		Variability	SD@ Age 7	1.58	1.47	1.69		1.6	1.46	1.73		1.66	1.54	1.77	
			SDSlope	0.02	0.02	0.03		0.02	0.02	0.03		0.02	0.02	0.03	
			Correlation(int,slope)	-0.5	-0.62	-0.38		-0.5	-0.64	-0.36		-0.33	-0.47	-0.19	

β= Beta co-efficient, SD = Standard Deviation
95%CI = 95% Confidence interval
P1 = Pubertal status 1, P2= Pubertal status 2, P3 = Pubertal status 3

Table 18 Linear mixed models for TSH FT3 and FT4 by pubertal status in girls (Tanner pubic hair domain) at age 13 years

				P1				P2				P3			
Parameter				β	95%CI		P Value	β	95%CI		P Value	β	95%CI		P Value
TSH	Girls	Main effects	Age 7	2.16	2.04	2.28	<0.001	2.28	2.17	2.39	<0.001	2.2	2.13	2.27	<0.001
			Slope	0.0004	-0.001	0.002	0.71	0.001	-0.001	0.003	0.21	0.001	-0.003	0.002	0.15
		Variability	SD@ Age 7	0.86	0.76	0.94		0.95	0.87	1.02		0.92	0.88	0.97	
			SDSlope	0.01	0.01	0.01		0.01	0.01	0.01		0.01	0.01	0.01	
			Correlation(int,slope)	-0.6	-0.74	-0.45		-0.57	-0.7	-0.45		-0.52	-0.61	-0.44	
T3	Girls	Main effects	Age 7	6.27	6.19	6.36	<0.001	6.27	6.2	6.34	<0.001	6.37	6.32	6.41	<0.001
			Slope	-0.007	-0.009	-0.005	<0.001	-0.007	-0.008	-0.006	<0.001	-0.01	-0.01	-0.01	<0.001
		Variability	SD@ Age 7	0.6	0.54	0.66		0.63	0.57	0.68		0.62	0.59	0.65	
			SDSlope	0.008	0.007	0.01		0.008	0.006	0.009		0.009	0.008	0.01	
			Correlation(int,slope)	-0.77	-0.86	-0.68		-0.57	-0.7	-0.44		-0.6	-0.67	-0.52	
T4	Girls	Main effects	Age 7	15.9	15.6	16.2	<0.001	15.8	15.6	16	<0.001	15.9	15.8	16	<0.001
			Slope	-0.004	-0.008	0.0003	0.07	-0.002	-0.006	0.002	0.43	-0.004	-0.006	-0.001	0.005
		Variability	SD@ Age 7	1.88	1.68	2.06		1.79	1.64	1.93		1.77	1.68	1.86	
			SDSlope	0.02	0.02	0.02		0.02	0.02	0.03		0.02	0.02	0.03	
			Correlation(int,slope)	-0.42	-0.61	-0.23		-0.43	-0.58	-0.27		-0.43	-0.53	-0.33	

β= Beta co-efficient, SD = Standard Deviation
95%CI = 95% Confidence interval
P1 = Pubertal status 1, P2= Pubertal status 2, P3 = Pubertal status 3

4A.3.5 Relationship between TSH and serum thyroid hormone levels in children at ages 7 and 15 years

At age 7 years, TSH was weakly positively associated with FT_3 after adjusting for age sex, FT_4 and markers of social class and early life environment β (standardised) =0.03 (95%CI 0.001, 0.06) p=0.05 whereas TSH was clearly negatively associated with FT_4 β (standardised) =-0.07 (95%CI -0.10, -0.04) $p=3.49x10^{-05}$ (**Table 19**). A similar pattern was also observed at age 15 years even after adjusting for pubertal status with TSH positively associated with FT_3 β (standardised) =0.07 (95%CI 0.02, 0.13) p=0.01 and negatively associated with FT_4 β (standardised) =-0.13 (95%CI -0.19, -0.07) $p=5.16x10^{-06}$ (**Table 19**). FT_3 and FT_4 were positively associated with each other at age 7 years B (standardised) =0.27 (95%CI 0.24, 0.30) $p=1.12x10^{-14}$ and also at age 15 years, β (standardised) =0.19 (95%CI 0.12, 0.26) $p=4.23x10^{-07}$.

Table 19 Relationships between TSH and other thyroid hormone parameters (standardized) at age 7 and age 15

Model	FT3 (pmol/l)			FT4 (pmol/l)		
	β (standardised)	95%CI	p*	β (standardised)	95%CI	p*
Age 7 (N=4,442)						
Model 1	0.01	-0.02, 0.04	0.56	-0.07	-0.10, -0.04	$1.09x10^{-06}$
Model 2	0.03	0.001, 0.06	0.05	-0.08	-0.10, -0.05	$1.63x10^{-07}$
Model 3	0.03	0.001, 0.06	0.05	-0.07	-0.10, -0.04	$3.49x10^{-05}$
Age 15 (N =1,263)						
Model 1	0.06	0.01, 0.11	0.02	-0.08	-0.14, -0.03	0.003
Model 2	0.08	0.03, 0.12	0.002	-0.10	-0.15, -0.04	$4.17x10^{-05}$
Model 3	0.08	0.03, 0.13	0.001	-0.12	-0.17, -0.06	$3.38x10^{-06}$
Model 4	0.07	0.02, 0.13	0.01	-0.13,	-0.19, -0.07	$5.16x10^{-06}$

* Calculated using the Wald test B=Beta coefficient CI = Confidence interval
p = strength of evidence against the null hypothesis of no association
Model 1 adjusted for age and sex
Model 2 adjusted for Model 1 and other thyroid hormone parameters
Model 3 adjusted for Model 2 and markers of social class and early life environment (home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score)
Model 4 adjusted for Model 3 and tanner stage

4A.4 DISCUSSION

My analysis shows that there are substantial changes in the pituitary-thyroid axis over childhood. In particular, FT_3 changes much more over childhood than either TSH or FT_4. Levels of FT_3 at age 7 are high compared to adult values with almost 25% of children at age 7 years have a FT_3 level above the adult reference-range and although there is a substantial fall in FT_3 levels between age 7 years and age 15 years, over 10% of values at age 15 years are still above the adult reference-range. Of note, FT3 levels fall more rapidly in girls, so that by age 15 none are above the adult reference range. As a result, girls had a higher FT_3 than boys at age 7 years, but by age 15 years this had reversed with boys now having higher FT_3 levels. In contrast to FT3, changes in TSH and FT_4 levels were modest: FT4 values showed almost no change and were already largely within the adult range at age 7; TSH values showed a small rise (**Figures 9 and 10**)

In all models, there was a very strong negative correlation between hormone levels between age 7 and 15, this suggests that the substantial variability observed in childhood is reduced through puberty, with hormones levels converging to near adult reference values. Overall my data suggests that there may be higher conversion of FT_4 to FT_3 in younger children than adults. My observation that boys maintain a higher FT_3 for longer than girls is also noteworthy and may have substantial importance in observed sex differences in bone development [198].

The reason why children have higher FT_3 levels at age 7 years is unclear but may be due to external factors to the pituitary-thyroid axis such as fat mas, growth hormone and pubertal development. In this analysis I observed that children that reached puberty earlier (as indicated by more advanced self-reported pubertal stage at age 13 years) had higher FT_3 values at age 7 years and a negative FT_3 slope between ages 7 and 15 years whereas those with less advanced puberty had a positive FT_3 slope between ages 7 and 15 years. This is also in keeping with our observation that girls having a higher FT_3 than boys at age 7 years with a greater fall in between ages 7 and 15 years.

These changes are more marked in girls than in boys, such that by age 15, although girls have a higher proportion of fat mass than boys at this age, FT_3 levels are lower. Our observed changes may also be partly due to thyroid derived changes in preparation for puberty, or as a consequence of other factors such as growth hormone, as growth hormone therapy has been linked to marginally increased FT_3 and decreased FT_4 levels [199].

Further changes in FT_3 must occur beyond age 15 especially in boys and those with later puberty onset, resulting finally in values within the adult reference range, and indeed it appears that falls in FT_3 occur even later in adult life [200] in males which may have implications for thyroid hormone replacement and treatment targets in children.

These findings are also clinically relevant, given the striking differences observed in early childhood thyroid hormone levels from adult derived reference-ranges. If age and sex appropriate reference ranges are not used, there may be substantial under-diagnosis of sub-clinical thyroid disease in children. In addition, the finding that children have substantially higher FT_3 levels than adults has implications for thyroid hormone replacement in congenital hypothyroidism. Individuals on levothyroxine have a higher FT_4 and a lower FT_3 than euthyroid individuals despite having similar TSH levels [185, 186]. Children on levothyroxine might therefore have inadequate FT_3 levels for optimal development and this merits further study particularly as FT_3 levels may have a more important role in both the assessment and therapy of thyroid disease than previously assumed [201]. The relative lack of FT_3 in these children may potentially be one of the reasons that optimal IQ levels are not reached in children with congenital hypothyroidism despite adequate levothyroxine therapy [202]. Taken together, there remains a pressing need for further study of central and peripheral determinants of thyroid function as well as determinants of intracellular thyroid status.

Strengths of this analysis include the use of a large population birth cohort with detailed phenotypic and genetic data available with paired thyroid function in a substantial number of individuals which allows more robust analysis than those performed from cross-sectional convenience samples. The nature of the cohort means it is unlikely that interfering

medications or heterophilic antibodies have influenced results. Furthermore, our use of liner mixed models has allowed us to determine the change of TSH and thyroid hormone levels between ages 7 and 15, whilst simultaneously adjusting for an individual's baseline hormone levels, indication this approach allows us to investigate how variability reduces as children progress into adulthood. Limitations of this analysis include that only 2 time points were used more thyroid function at ages 5, 11 and 18 years might enable us to better understand the trajectory of thyroid function over childhood. A higher social class bias in our dataset and lack of generalizability to ethnic minorities as 98% of all samples analyzed were in individuals of Caucasian descent. Furthermore all individuals were from a small region of the UK which has been shown to be borderline iodine deficient [203]. Pubic hair domain of the Tanner stage could only be used as other domains were unreliable with some individuals appearing to regress over puberty; this may be due to the fact that parents completed these pubertal assessments in a written questionnaire. Our findings require replication in individuals from other ethnic groups and using different thyroid hormone assays. Ideally TPO antibody levels should have been measured to perform reference range analysis in individuals who had no evidence of underlying autoimmune thyroid disease, however TPO antibodies were unavailable in this cohort although the number of children with substantial thyroid disease from TPO positivity are likely to be small.

In summary, this part of the chapter demonstrates that thyroid hormone levels change substantially during childhood, in particular FT_3 which is substantially higher in younger children and has a different relationship to FT4 with TSH. This is the first analysis to utilize longitudinal data from a population birth cohort rather than cross-sectional convenience samples enabling us to explore the trends in TSH and thyroid hormone levels and their inter-relationships in childhood and for the first time study how their longitudinal stability relates to pubertal status.

Taken together, these data suggests that the regulation and the role of FT3 may be different from that of FT4. It appears that the regulation on FT4 is primarily regulated by the HPT axis although FT3 appears to be more fluid. This FT3 fluidity is also observed in sick euthyroidism, which is observed in unwell individuals where FT3 levels tend to be low, with relative preservation of TSH and FT4. This work also raises the issue that the primary roles of FT3 and FT4 may be different as FT4 is tightly regulated by the HPT axis, whereas FT3 is influenced by other factors. From this analysis, it is unclear whether the FT3 rise in very early puberty is necessary for pubertal progression or simply a response to other factors such as changes in growth hormone levels. The original observation in my initial analyses that FT3 and FT4 had a different association with fat mass with FT3 being surprisingly positively associated with fat mass. Studying this relationship between thyroid status and body composition will be the focus of the next part of this chapter (**4B**).

4B.1 INTRODUCTION

The relationship between pathological thyroid dysfunction and body composition is well established, with hyperthyroidism associated with weight loss and hypothyroidism associated with weight gain [204, 205]. As I observed in the meta-analysis undertaken earlier in this thesis (chapter 3) higher levels of TSH were associated with higher levels of BMI. Although this relationship has not been studied in children. Longitudinal studies have highlighted that weight gain is associated with an increase in TSH levels [145] whereas weight loss is related to decreased TSH and also surprisingly to decreased FT3 levels [206]. Cross-sectional studies have indicated that FT3 is positively associated with BMI and fat mass whereas FT4 is negatively associated [155, 207-209].

In this section of the thesis I will explore the relationship between thyroid status and body composition using data from ALSPAC.

4B.2 METHODS

4B.2.1 Study participants and phenotypes

The ALSPAC cohort has already been described in detail in chapter 2 BMI was calculated as weight (in kilograms) divided by height (in meters) squared. As a measure of adiposity BMI has several limitations [210]; in particular, it does not reliably distinguish between fat and lean mass. Therefore, total fat mass was also assessed using a Lunar Prodigy narrow fan beam densitometer to perform a whole-body dual-energy x-ray absorptiometry (DXA) scan. Additional details of these measurements,

including their reproducibility, are described elsewhere [211]. The fat mass index (FMI) was also calculated as total fat mass (in kilograms) divided by height (in meters) squared. Data on BMI were collected at ages 7 and 15 years with anthropometric data from DXA performed at ages 9.9 and 15.5 years.

4B.2.2 Statistical Analysis

Implausible height, weight, BMI, fat mass, lean mass, blood pressure and thyroid measurement (>4 SD from the mean for the sex and age-specific category) were considered as outliers and recoded to missing. TSH and fat mass were natural log transformed to approximate the normal distribution. Descriptive statistics are presented as backtransformed (geometric) means, standard deviations (SD), medians and lower and upper quartiles. All thyroid, body composition and blood pressure variables were standardized; analyses are therefore presented as per SD.

Analyses were adjusted for several key confounders these included child derived variables – sex of child, age of child at assessments in months and thyroid. Due to the intimate relationship between social class and home environment on weight and body composition analyses were adjusted for maternal age at birth of child, parity of mother at birth of child, maternal smoking during pregnancy, educational status of mother, markers of affluence including housing status, family adversity index and HOME score. Further details of these confounders are available in Appendix 1.

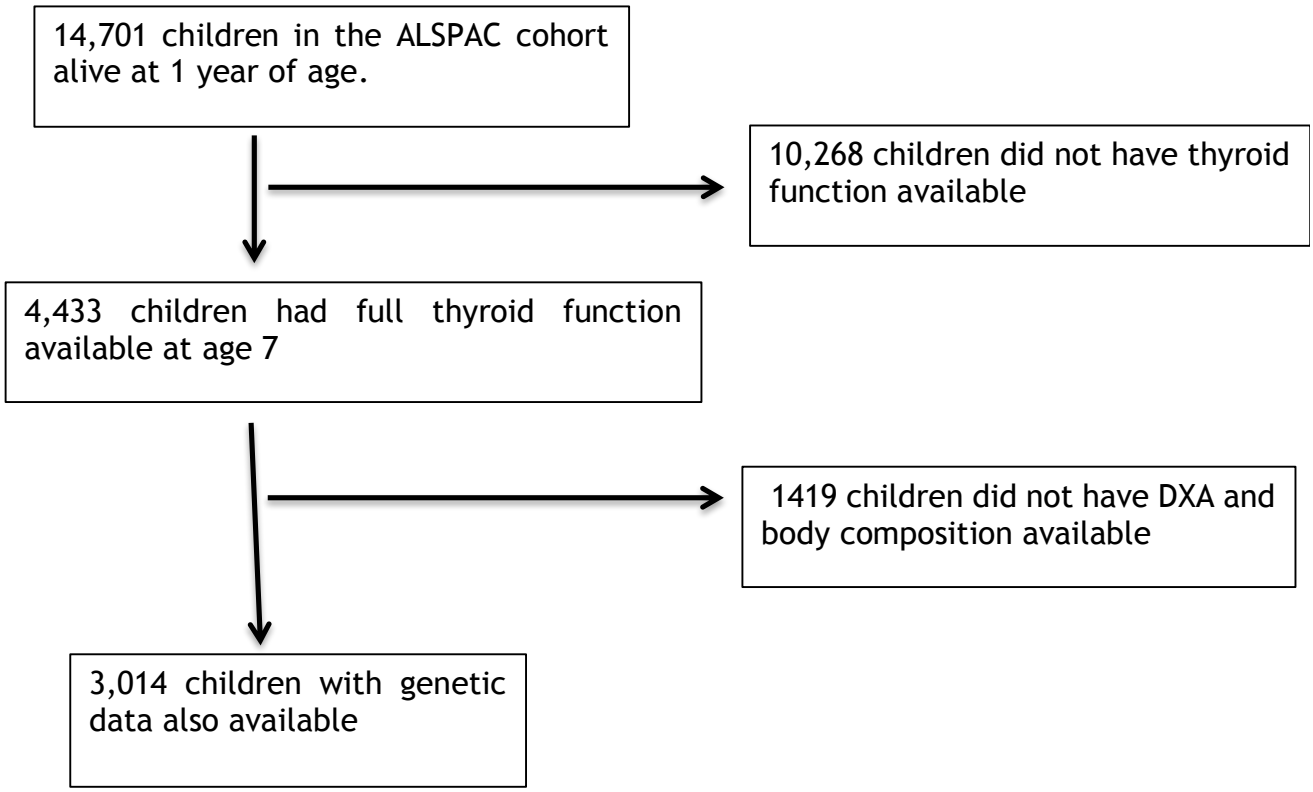
Analyses were initially performed adjusted for child's age at measurements and sex (model 1). Sex is likely to be associated with differences in thyroid function and body composition so is a key confounder. Age at thyroid measurements may influence thyroid status although little is known regarding this. Where appropriate, three further models controlling for key potential confounders were undertaken. These were selected markers of social class. The ALSPAC cohort is borderline iodine deficient[203], social class may influence diet particularly iodine in the diet and in turn has a key impact on body composition so this is important to include in models Model 2 also adjusted for thyroid hormone parameters, model 3=model 2 adjusted for height, measures of social class and early life environment including home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score listed below. Height was included in model 3 as although BMI was designed to assess weight independently of height, it remains correlated with height owing to its generalized derivation. Analysis of anthropometric relationships with thyroid hormone status at age 15 also incorporated pubertal status (model 4) which was assessed using a Tanner stage questionnaire [198] at age 13.5 years (pubic hair domain) range from 13.1 to 14.4 years.

4B.3 RESULTS

4B.3.1 Study population and baseline characteristics

3,014 individuals (1,542 males, 1,472 females) had relevant thyroid, body composition and other phenotypic data available (**Figure 11**).

Figure 11 Study participants for thyroid and body composition analysis



473 (16.6%) of children in our study population were obese or overweight at age 7; 373 were classified as overweight with 100 classified as obese according to cutoffs proposed by the International Obesity Task Force [212].

4B.3.2 Observational analysis of baseline characteristics

There was a good correlation between BMI at age 7 years and FMI at age 9 years (Pearson's correlation coefficient=0.73). Height and lean mass were both greater in males (126.5cm vs. 125.7cm $p<0.001$) (25.6Kg vs.

23.7Kg p<0.001) respectively, however BMI and fat mass were greater in females (16.0kg/m^2 vs. 16.3kg/m^2 p <0.001) (9.46kg vs. 7.10kg p<0.001). Baseline characteristics are shown in **Table 20**

Table 20 Baseline characteristics for body composition analysis

Variable	All (N=3,014) Mean or percentage	SD	Males (N=1,542) Mean or percentage	SD	Females (N=1,472) Mean or percentage	SD
BMI age 7 (Kg/m2)	16.1	1.89	16.0	1.76	16.3	2.09
Height age 7 (cm)	126.1	5.61	126.5	5.56	125.7	5.64
Weight age 7 (kg)	25.8	4.34	25.7	4.16	25.9	4.51
Fat mass measured by DXA (kg)	8.26	4.77	7.10	4.53	9.46	4.71
Body fat percentage [fat mass (kg)/weight (kg)]x100[1]	22.6	8.69	19.4	18.26	25.9	7.87
Lean mass measured by DXA (kg)	24.7	3.11	25.6	2.89	23.7	3.05
Age at DXA assessment (months)	90.3	3.92				
Age 7 FT3(pmol/liter)	6.28	0.62	6.22	0.62	6.34	0.62
Age 7 FT_4 (pmol/liter)	15.7	1.66	15.5	1.58	15.9	1.71
Age 7 TSH (mU/l)	2.28	0.91	2.36	0.90	2.20	0.90
Age 15 FT3(pmol/liter)	5.84	0.74	6.19	0.69	5.50	0.63
Age 15 FT_4 (pmol/liter)	15.4	1.90	15.4	1.98	15.4	1.84
Age 15 TSH (mU/l)	2.33	1.00	2.43	1.05	2.24	0.93
Family Adversity Index[2]	3.71	4.45	3.73	3.87	3.69	3.80
Home Score3						
0-4 (%)	3.47		2.77		4.21	
5-8 (%)	23.9		23.2		24.6	
9-12 (%)	72.7		74.1		71.2	
Housing Status[4]						
Owned/mortgaged (%)	85.6		85.6		85.6	
Privately rented (%)	11.0		11.8		10.2	
Council rented/other (%)	3.3		2.7		4.0	
Maternal age at childbirth (years)	29.4	4.2	29.5	4.5	29.4	4.3
Maternal highest educational status[5]						
Low (%)	18.4		19.3		17.4	
Middle (%)	34.8		35.5		34.1	
High (%)	46.8		45.2		48.5	
Maternal smoking in pregnancy[6]						
None (%)	84.5		84.3		84.7	
Some (%)	15.5		15.7		15.2	
Parity[7]						
0 - 1 (%)	82.1		80.9		83.4	
2 – 4 (%)	17.9		18.7		16.6	
> 5 (%)	0.2		0.28		0.07	

[1] 2 children with missing data for weight at age 9, [2] 323 children with missing data, [3] 277 children with missing data, [4] 215 children with missing data, [5] 224 children with missing data, [6] 201 children with missing data, [7] 226 children with missing data.

4B.3.3 Relationship between thyroid status and body composition

BMI at age 7 years was positively associated with FT3 at age 7 years Beta (ß) standardized (standardised)=0.13 (95%CI 0.10, 0.16) $p=5.3x10^{-16}$ and fat mass at age 9 ß (standardised)=0.18 (95%CI: 0.15, 0.21) $p=4.3x10^{-25}$ even after adjustment for confounders (**Table 21**). FT3 was also positively associated with lean mass ß (standardised)=0.10 (95%CI: 0.06, 0.13) $p=6.6x10^{-09}$. However, adding fat mass to this model attenuated the association (ß (standardised)=0.02, 95%CI: -0.02, 0.04 p=0.32). In contrast adjusting for lean mass in the relationship between FT3 and BMI and FT3and fat mass had no substantial effect on effect estimates ß (standardised)=0.07 (95%CI: 0.04, 0.10) $p=7.7x10^{-07}$ and ß (standardised)=0.12 (95%CI: 0.09, 0.16) $p=5.7x10^{-14}$ respectively.

Table 21 Associations between measures of adiposity and thyroid hormone parameters at age 7

	N	TSH (mU/l) β (std)	95%CI	p*	FT3 pmol/liter B (std)	95%CI	p*	FT4 pmol/liter β (std)	95%CI	p*	FT3FT4 Ratio# β(std)	95%CI	p*
		BMI (age 7)											
Model 1	3,014	0.03	0.004, 0.06	0.05	0.13	0.10, 0.16	$5.3x10^{-16}$	-0.05	-0.08, -0.02	0.001	0.15	0.12, 0.18	$1.4x10^{-20}$
Model 2	3,014	0.02	-0.01, 0.06	0.13	0.16	0.13, 0.19	$6.6x10^{-21}$	-0.10	-0.13, -0.06	$1.6x10^{-08}$	-	-	-
Model 3	3,014	0.03	-0.004,0.07	0.09	0.12	0.08, 0.16	$4.0x10^{-10}$	-0.08	-0.12, -0.04	0.00003	0.12	0.09, 0.16	$5.7x10^{-11}$
		Weight (age 7)											
Model 1	3,014	0.02	-0.01, 0.05	0.15	0.11	0.08, 0.14	$8.1x10^{-17}$	-0.04	-0.07, 0.02	0.001	0.13	0.10, 0.15	$8.4x10^{-22}$
Model 2	3,014	0.01	-0.01, 0.04	0.35	0.13	0.11, 0.16	$5.2x10^{-22}$	-0.08	-0.11, -0.05	$6.3x10^{-09}$	-	-	-
Model 3	3,014	0.02	-0.001, 0.05	0.08	0.09	0.06, 0.13	$5.6x10^{-10}$	-0.05	-0.08 -0.03	0.0001	0.08	0.06, 0.11	$2.0x10^{-07}$
		Fat mass (age 9)											
Model 1	3,014	0.01	-0.02, 0.04	0.74	0.18	0.15, 0.21	$4.3x10^{-25}$	-0.04	-0.08, -0.01	0.02	0.18	0.15, 0.21	$1.8x10^{-25}$
Model 2	3,014	-0.003	-0.03, 0.03	0.87	0.21	0.17, 0.24	$1.1x10^{-30}$	-0.10	-0.13, -0.06	$2.6x10^{-08}$	-	-	-
Model 3†	2,538	-0.008	-0.04, 0.03	0.64	0.12	0.09, 0.16	$4.0x10^{-12}$	-0.07	-0.10, -0.03	0.002	0.11	0.08, 0.14	$8.0\ x10^{-11}$
		FMI (age 9)											
Model 1	3,014	0.01	-0.03, 0.04	0.75	0.17	0.13, 0.20	$1.5x10^{-22}$	-0.04	-0.07, 0.002	0.04	0.17	0.14, 0.20	$1.5x10^{-22}$
Model 2	3,014	-0.003	-0.04, 0.03	0.84	0.19	0.16, 0.23	$2.1x10^{-27}$	-0.10	-0.13, -0.06	$2.4x10^{-07}$	-	-	-
Model 3†	2,538	-0.01	-0.04, 0.02	0.65	0.13	0.09, 017	$3.9x10^{-12}$	-0.07	-0.11, -0.03	0.002	0.12	0.08, 0.15	$7.9x10^{-11}$
		Lean mass (age 9)											
Model 1	3,014	0.01	-0.02, 0.06	0.18	0.10	0.06, 0.13	$6.6x10^{-09}$	-0.05	-0.09, -0.02	0.002	0.12	0.09, 0.16	$1.7x10^{-13}$
Model 2	3,014	0.02	-0.02, 0.05	0.35	0.12	0.09, 0.15	$3.4x10^{-12}$	-0.09	-0.12, -0.05	$8.9x10^{-07}$	-	-	-
Model 3†	2,538	0.02	-0.01, 0.04	0.11	0.01	-0.02, 0.03	0.56	-0.03	-0.05,-0.01	0.01	0.02	-0.0001, 0.04	0.05

*Calculated using the Wald test † 476 individuals with missing data # Not adjusted for other thyroid hormone parameters N=Number B=Beta coefficient CI = Confidence interval p = strength of evidence against the null hypothesis of no association std=standardised Model 1 adjusted for age and sex Model 2 adjusted for Model 1 and other thyroid hormone parameters Model 3 adjusted for Model 2 and height, markers of social class and early life environment (home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score)

FT4 was negatively associated with fat mass ß (standardised)=-0.04 (95%CI: -0.08, -0.01) p=0.02 and lean mass ß (standardised)=-0.05 (95%CI: -0.09, -0.02) p=0.002 as well as height and weight (**Table 21**). No clear associations between TSH and body composition were observed even after adjusting for confounders and other thyroid hormone parameters.

Similar cross-sectional associations were also observed with thyroid hormone parameters at age 15 years and BMI at age 15 years for FT3and FT_3:FT_4 ratio, however effect estimates were weaker (**Table 22**). Much weaker or no associations were observed with other components of body composition. Analysis revealed that for every 0.20 kg/m^2 increase in FMI between ages 9 and 15 years there was a 0.10pmol/l increase in FT3(p=0.005).

Table 22 Relationship between thyroid hormone parameters and body composition at age 15 (N=730)

	TSH (mU/l)			FT3 pmol/liter			FT4 pmol/liter			FT3FT4 Ratio#		
	β (std)	95%CI	p*	β (std)	95%CI	p*	β (std)	95%CI	p*	β (std)	95%CI	p*
	BMI (age 15)											
Model 1	0.02	-0.05, 0.08	0.62	0.07	-0.01, 0.14	0.09	-0.05	-0.12, 0.01	0.13	0.10	0.03, 0.17	0.009
Model 2	0.005	-0.07, 0.08	0.89	0.08	-0.001, 0.16	0.05	-0.07	-0.14, 0.01	0.07	-	-	-
Model 3	0.02	-0.05, 0.09	0.64	0.07	-0.01, 0.15	0.10	-0.05	-0.12, 0.03	0.21	0.08	0.01, 0.17	0.03
Model 4	-0.02	-0.10, 0.06	0.60	0.09	-0.001, 0.19	0.05	-0.07	-0.15, 0.01	0.08	0.11	0.02, 0.19	0.02
	Weight (age 15)											
Model 1	0.01	-0.06, 0.07	0.85	0.02	-0.05, 0.10	0.54	-0.01	-0.08, 0.05	0.69	0.03	-0.04, 0.10	0.43
Model 2	0.002	-0.07, 0.07	0.94	0.03	-0.05, 0.10	0.50	-0.02	-0.09, 0.05	0.62	-	-	-
Model 3	0.01	-0.06, 0.07	0.79	0.03	-0.05, 0.11	0.54	-0.004	-0.07, 0.07	0.91	0.02	-0.05, 0.10	0.53
Model 4	-0.03	-0.10, 0.05	0.46	0.06	-0.03, 0.14	0.20	-0.05	-0.13, 0.02	0.17	0.07	-0.01, 0.14	0.11
	Height (age 15)											
Model 1	-0.01	-0.07, 0.04	0.62	-0.07	-0.13, 0.002	0.04	0.06	0.004, 0.12	0.03	-0.11	-0.17, -0.05	0.01
Model 2	-0.001	-0.06, 0.06	0.98	-0.08	-0.14, -0.01	0.02	0.07	0.02, 0.13	0.01	-	-	-
Model 3†	-0.002	-0.06, 0.06	0.95	-0.07	-0.13, 0.001	0.05	0.07	0.01, 0.12	0.03	-0.10	-0.15, -0.03	0.004
Model 4††	-0.01	-0.07, 0.06	0.84	-0.04	-0.11, 0.03	0.30	0.01	-0.06, 0.08	0.79	-0.04	-0.11, 0.03	0.26
	Fat mass (age 15)											
Model 1	0.01	-0.05, 0.07	0.72	0.03	-0.03, 0.10	0.32	-0.03	-0.09, 0.03	0.33	0.06	-0.003, 0.12	0.06
Model 2	0.004	-0.06, 0.07	0.89	0.04	-0.03, 0.11	0.25	-0.03	-0.09, 0.03	0.35	-	-	-
Model 3†	0.01	-0.05, 0.07	0.80	0.03	-0.03, 0.10	0.38	-0.03	-0.10, 0.03	0.30	0.06	-0.01, 0.12	0.08
Model 4††	-0.01	-0.08, 0.06	0.70	0.04	-0.05, 0.12	0.37	-0.06	-0.13, 0.01	0.10	0.07	-0.002, 0.15	0.06
	FMI (age 15)											
Model 1	0.01	-0.04, 0.07	0.66	0.04	-0.02, 0.10	0.19	-0.04	-0.09, 0.02	0.19	0.07	0.01, 0.13	0.02
Model 2	0.004	-0.05, 0.06	0.88	0.05	-0.01, 0.12	0.13	-0.04	-0.10, 0.01	0.13	-	-	-
Model 3†	0.01	-0.05, 0.07	0.79	0.04	-0.03, 0.11	0.24	-0.04	-0.10, 0.02	0.17	0.07	0.01, 0.13	0.03
Model 4††	-0.01	-0.08, 0.05	0.72	0.04	-0.04, 0.12	0.29	-0.06	-0.13, 0.01	0.10	0.08	0.005, 0.15	0.04
	Lean mass (age 15)											
Model 1	-0.005	-0.05, 0.04	0.82	-0.04	-0.09, 0.01	0.09	0.02	-0.02, 0.06	0.41	-0.05	-0.10, -0.01	0.02
Model 2	0.001	-0.04, 0.05	0.95	-0.05	-0.10, 0.003	0.07	0.02	-0.03, 0.06	0.42	-	-	-
Model 3†	0.002	-0.04, 0.05	0.93	-0.04	-0.09, 0.02	0.16	0.03	-0.02, 0.07	0.21	-0.05	-0.09, -0.003	0.05
Model 4††	-0.02	-0.07, 0.03	0.41	0.01	-0.05, 0.07	0.80	-0.01	-0.07, 0.04	0.71	-0.002	-0.06, 0.05	0.93

† 51 individuals with missing data †† 202 individuals with missing data B=Beta coefficient CI = Confidence interval, std = standardized p = strength of evidence against the null hypothesis of no association Model 1 adjusted for age and sex Model 2 adjusted for Model 1 and other thyroid hormone parameters Model 3 adjusted for Model 2 and height, markers of social class and early life environment (home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score) Model 4 adjusted for Model 3 and Tanner stage

4B.4 DISCUSSION

My observational analyses clearly show that FT3 and FT_4 have opposing strong correlations with body composition in childhood; with FT3 being positively associated and FT_4 negatively associated with fat mass and BMI. This effect persisted after adjusting for key confounders. Even residual confounding would be unlikely to explain such striking opposite relationships between FT3 and FT4 on body composition. Furthermore, this effect appeared to be more pronounced at age 7, an age when we also observed substantially higher FT3 levels.

This finding is in keeping with other smaller recent studies have also shown that in healthy euthyroid adults FT_3 is positively associated with BMI and measures of adiposity whereas FT_4 is negatively associated [155, 207-209]. It is also in keeping with the observation that obese individuals have higher FT3 levels [207, 213] although other studies have reported higher FT4 levels as well in morbidly obese patients[214].

The nature of our cross-sectional analysis is such that we cannot determine whether higher FT3 levels in children result in higher fat mass through its effect on growth or whether higher levels of fat mass either increase FT3 levels directly or via a response from the HPT axis. This is an important issue to address as the nature of the FT3 fat mass relationship has substantial implications for our understanding of the thyroid axis as well as body composition.

To explore this relationship further I undertook additional instrumental variable analyses to better clarify the relationship between FT3 and fat mass, this is described in the next part of the chapter (**4C**).

4C.1 INTRODUCTION

My observed positive association between FT3 and BMI/fat mass were derived from cross-sectional analyses and therefore vulnerable to reverse causation and confounding. An alternative approach is to investigate the effect of genetic variation in levels of the exposure on the outcome of interest, this approach is widely known as Mendelian Randomization (MR) [215]. The genetic architecture of BMI is well understood [216], however whilst TSH and to a lesser extent FT4 have been studied there has been no genome-wide association studies of FT3 to date [217, 218]. Therefore the approach I will use is to see if genetic variation in variants associated with BMI is associated with changes in FT3 levels. This analytical approach may enable us to clarify the nature of the relationship between body mass index and FT3 and assess whether changes in BMI /fat mass causally affect thyroid status.

The use of MR in analyses is based on the random allocation of genetic variants from parents to offspring. This random allocation means that these variants will generally be unrelated to other factors which affect the outcome of interest [219]. The MR approach applies instrumental variable methods, using genetic variants as a proxy for the exposure of interest. In this method, the use of genetic instruments associated with BMI confers several advantages above traditional regression analysis [215, 219, 220]. First, the direction of causation is from the BMI genetic instruments onto FT_3, and cannot be due to reverse causation as FT3 levels cannot cause genetic variation. Second, BMI is associated with a

wide range of behavioural, social and physiological factors that might confound its association with FT3. The use of genetic variants associated with BMI substantially reduces the risk of this confounding and they can be regarded as an unconfounded indicators [221]. Third, genetic variants and their effects are subject to relatively little measurement error or bias.

As alleles are largely passed from parents to offspring independently of the environment, offspring who inherit more alleles associated with BMI are in effect being randomly assigned a higher BMI dosage. Several assumptions need to be met, firstly that the genotype is associated with the exposure, secondly that the genotype is associated with the outcome through the studied exposure only (exclusion restriction assumption), third that the genotype is independent of other factors which may influence the outcome (independence assumption). However, there are potential violations that can occur which can negate these assumptions[219]. These include i) Population stratification - the presence of a systematic difference in allele frequencies between subpopulations. ii) Linkage disequilibrium – the non-random association of alleles at different loci in a given population which can lead to spurious associations. iii) Pleiotropic effects – where when one variant influences two or more seemingly unrelated phenotypic traits. iv) Canalisation -the ability of a population to produce the same phenotype regardless of variability of its environment or genotype

With the above caveats, MR can therefore be thought of as analogous to a randomized trial with randomization by genotype taking place at conception. A by-genotype analysis is equivalent to an intention-to-treat analysis in a randomized controlled trial, in which individuals are analysed according to the group they were randomized into, independent of whether they complied to the treatment regimen or not.

In this section of the thesis, I now undertook MR analyses to examine the nature of the relationship between BMI and FT_3. I used 32 independent genetic correlates of BMI, confirmed in a large-scale meta-analysis of genome-wide association studies [216], to assess whether there is a causal pathway between BMI and FT3 levels which would explain the paradoxical opposing relationship between FT3and FT_4 on BMI in observational studies.

4C.2. METHODS

Details on the ALSPAC cohort have been described in chapter 2 and laboratory measures and DXA scans have been described in detail earlier in this chapter.

4C.2.1Genotyping

Genotyping in ALSPAC has been previously described [222]. GWAS data was generated by Sample Logistics and Genotyping Facilities at the Wellcome Trust Sanger Institute and LabCorp (Laboratory Corportation of America) using support from 23andMe. Single nucleotide polymorphisms (SNPs) were removed if the minor allele frequency (MAF) was <1%, the

call rate was <95% or the p-value from an exact test of Hardy-Weinberg equilibrium was $<5.7x10^{-7}$. Individual samples were excluded on the basis of incorrect sex assignment, minimal or excessive heterozygosity, high levels of missingness and cryptic relatedness (16%). Established BMI variants that had not been genotyped directly were imputed with MACH 1.0.16 Markov Chain Haplotyping software [223, 224] using CEPH individuals from HapMap phase 2 (release 22) reference set.

4C.2.2 Instrumental variable analysis

Speliotes et al. previously reported 32 genetic variants to be robustlt associated with BMI [216]. These are shown in **Table 23**.

Table 23 Details of SNPs associated with adiposity in a large GWAS meta-analysis by Speliotes et al

SNP	Nearest Gene	Chromosome	Base pair position	Coefficient from GWAS for weighting
rs1514175	TNNI3K	1	74,764,232	0.07
rs1555543	PTBP2	1	96,717,385	0.06
rs2815752	NEGR1	1	72,585,028	0.13
rs543874	SEC16B	1	176,156,103	0.22
rs2867125	TMEM18	2	612,827	0.31
rs2890652	LRP1B	2	142,676,401	0.09
rs713586	RBJ	2	25,011,512	0.14
rs887912	FANCL	2	59,156,381	0.1
rs13078807	CADM2	3	85,966,840	0.1
rs9816226	ETV5	3	187,317,193	0.14
rs10938397	GNPDA2	4	44,877,284	0.18
rs13107325	SLC39A8	4	103,407,732	0.19
rs2112347	FLJ35779	5	75,050,998	0.1
rs4836133	ZNF608	5	124,360,002	0.07
rs206936	NUDT3	6	34,410,847	0.06
rs987237	TFAP2B	6	50,911,009	0.13
rs10968576	LRRN6C	9	28,404,339	0.11
rs10767664	BDNF	11	27,682,562	0.19
rs3817334	MTCH2	11	47,607,569	0.06
rs4929949	RPL27A	11	8,561,169	0.06
rs7138803	FAIM2	12	48,533,735	0.12
rs4771122	MTIF3	13	26,918,180	0.09
rs10150332	NRXN3	14	79,006,717	0.13
rs11847697	PRKD1	14	29,584,863	0.17
rs2241423	MAP2K5	15	65,873,892	0.13
rs12444979	GPRC5B	16	19,841,101	0.17
rs1558902	FTO	16	52,361,075	0.39
rs7359397	SH2B1	16	28,793,160	0.15
rs571312	MC4R	18	55,990,749	0.23
rs2287019	QPCTL	19	50,894,012	0.15
rs29941	KCTD15	19	39,001,372	0.06
rs3810291	TMEM160	19	52,260,843	0.09

SNP = Single nucleotide polymorphism

An ‘allelic score’ was then created by summing the dosages for BMI-increasing alleles across all 32 SNPs [225]. Here the dose of the effect allele at each locus was weighted by the effect size of the variant in this independent meta-analysis [216]; these doses were then summed to

reflect the average number of effect weighted BMI increasing alleles carried by an individual. This combined score was able to explain a greater proportion of variance in BMI than single SNPs [226] and served as a genetic predictor in our MR analysis. This approach has previously been used in ALSPAC to study the relationship between adiposity and physical activity in children [227].

For investigating associations between the allelic score and standardized phenotypes, continuous effects were estimated using linear regression with adjustment for models as above. An additive genetic model was assumed since there was no evidence for interaction effects among the SNPs combined in the allelic score [216, 228]. Although co-variates are anticipated to be randomly distributed with respect to genotype [215] we also examined associations between confounders and genotypes to check the core instrumental variable assumption that the genetic instrument (BMI allelic score) is independent of factors that might potentially confound the observational association [219], and allow for comparison with conventional observational epidemiological models.

We then performed a two-stage least squares regression using the weighted allelic score as an instrument for BMI/adiposity using the "ivreg2" command in Stata. *F*-statistics from the first-stage regression between genotype and BMI/adiposity were examined to check the assumption that the instrument is sufficiently associated with the exposure to reduce the possibility of weak instrument bias [229]. Using

the “ivendog’ command in Stata the Durbin-Wu-Hausman (DWH) test for endogeneity was performed to compare effect estimates from the second stage of the instrumental variable analysis and estimates from linear regression. Models were repeated as described above.

4C.2.3 Sensitivity analyses

To explore the potential distorting effects of pleiotropy in our analysis we repeated our analyses using two independent genetic instruments. Pleiotropy occurs when a genetic instrument has an effect on the outcome (FT_3) independently of its effect on the exposure (adiposity), which has implications for key assumptions made in MR analyses [230]. Similar instrumental variable estimates acquired using two independent instruments would provide suggestive evidence against the existence of a pleiotropic effect, as it would be unlikely that both instruments had shared pleiotropy [230]. The two independent genetic instruments were rs1558902 in *FTO* (the individual SNP with the largest effect size on BMI identified by Speliotes et al. [216]), and a weighted allelic score constructed from the remaining 31 SNPs associated with BMI.

All data analysis was performed using Stata version 12.1 (STATACORP, College Station, TX).

4C.3 RESULTS

4C.3.1 Genotypic associations

The “allelic score” for BMI was normally distributed with a mean of 29.1 (SD 3.87 range 15.1–45.0) and explained 2.29% of the variance in standardized BMI and 2.93% of the variance in standardized FMI.

A per allele change in allelic score was associated with a 0.04 (95%CI: 0.03, 0.04) SD increase in BMI ($p=6.41\times10^{-17}$) at age 7 years, a 0.04 SD increase (95%CI: 0.04, 0.05) in FMI ($p=4.87x10^{-21}$) at age 9 years and a 0.05 SD increase (95%CI: 0.04, 0.06) in BMI at age 15 years ($p=1.16x10^{-16}$). In contrast to measures of adiposity, confounding factors were not associated with the allelic score in this cohort aside from a weak association for maternal smoking in pregnancy, likely representing a type-1 error (**Table 24**).

Table 24 Associations between the weighted allelic score for 32 SNPs and potential confounders at ages 7 and 15 years

Confounders	Per allele effects				
	β	95%CI	β (std)	95%CI	p*
FT_4 (age 7)	0.0003	-0.02, 0.02	0.0002	-0.01, 0.01	0.97
TSH (age 7)	-0.003	-0.01, 0.001	-0.01	-0.02, 0.001	0.15
FT_4 (age 15)[1]	-0.007	-0.04, 0.03	-0.004	-0.02, 0.02	0.69
TSH (age 15)[1]	-0.009	-0.02, 0.0002	-0.02	-0.04, 0.0005	0.06
Maternal agegroup	0.01	-0.005, 0.008	-	-	0.66
Maternal education[2]	-0.005	-0.02, 0.07	-	-	0.43
Parity[3]	0.008	-0.002, 0.005	-	-	0.67
Maternal smoking[4]	0.004	0.001, 0.007	-	-	0.04
FAI[5]	-0.02	-0.05, 0.02	-	-	0.38
Own home[6]	0.002	-0.003, 0.006	-	-	0.48
Parents and home score[7]	0.004	-0.001, 0.009	-	-	0.15

*Calculated using the Wald test
β=Beta coefficient
CI = Confidence interval
p = strength of evidence against the null hypothesis of no association
[1] 2,279 children with missing data, [2] 224 children with missing data [3] 226 children with missing data [4] 201 children with missing data [5]323 children with missing data [6] 215 children with missing data [7] 277 children with missing data

4C.3.2 Mendelian Randomization

Genetic analysis demonstrated that a higher BMI of 1 SD (1.89kg/m2) at age 7 years was associated with a 0.22 pmol/liter (95% CI, 0.07, 0.36) increase in FT3 (p=0.004) (**Table 25**). We did not observe strong evidence of a departure of instrumental-variable-derived estimates from observational results, as demonstrated by DWH tests (p=0.08), indicating similarity between observational and MR estimates in the effect of BMI on FT3. Point estimates for standardized effect sizes from the instrumental variable analysis were greater (0.30 vs. 0.13) than those derived from basic observational analyses. Similar results were observed when FMI at age 9 years was instrumented (**Table 25**). In contrast there was no substantial evidence of an association with FT_4 ß (standardised)=0.01 (95%CI: -0.23, 0.24) p=0.96 (**Table 25**). The higher

β observed with genetic rather than traditional analysis might be explained by FT3 having a negative effect on fat mass (which was originally expected) however the effect of fat mass on FT3 is far greater thus resulting in a positive association.

Table 25 Associations between body mass index/fat mass index and FT3and FT4 levels at age 7 as tested both by conventional epidemiological approaches and through the application of instrumental variable analysis using a 32-SNP weighted allelic BMI score

Adiposity	Model	N	Observational Linear Regression			Instrumental Variable Regression (Weighted Allelic Score with 32 SNPs)					
			β (95% CI)	β (std) (95% CI)	p	*F*-Statistic	Partial *R*2	β (95% CI)	B(std) (95% CI)	p	p (DWH)
FT3age 7											
BMI(kg/m2)	Model 1	3,014	0.05 (0.04, 0.06)	0.13 (0.10, 0.16)	$5.3x10^{-16}$	72.4	0.009	0.22 (0.07, 0.36)	0.35 (0.11, 0.58)	0.004	0.08
Age 7	Model 2	3,014	0.05 (0.04, 0.06)	0.16 (0.13, 0.19)	$6.6x10^{-21}$	73.7	0.009	0.22 (0.08, 0.36)	0.35 (0.143 0.57)	0.002	0.09
	Model 3†	2,538	0.04 (0.03, 0.05)	0.12 (0.08, 0.16)	$4.0x10^{-13}$	56.6	0.08	0.24 (0.06, 0.42)	0.38 (0.09, 0.68)	0.01	0.06
FMI(kg/m2)	Model 1	3,014	0.22 (0.17, 0.26)	0.17 (0.13 0.20)	$1.5x10^{-22}$	100.4	0.03	0.19 (0.07, 0.31)	0.30 (0.10, 0.50)	0.002	0.21
Age 9	Model 2	3,014	0.23 (0.19, 0.27)	0.19 (0.16, 0.23)	$2.1x10^{-27}$	100.7	0.11	0.19 (0.08, 0.31)	0.31 (0.12, 0.50)	0.001	0.22
	Model 3†	2,538	0.17 (0.12, 0.22)	0.13 (0.09, 0.17)	$3.9x10^{-12}$	79.5	0.09	0.20 (0.05, 0.35)	0.32 (0.08, 0.56)	0.008	0.14
FT4 age 7											
BMI(kg/m2)	Model 1	3,014	-0.07 (-0.10, -0.03)	-0.05 (-0.08, -0.02)	0.001	72.4	0.01	0.01 (-0.37, 0.40)	0.01 (-0.23, 0.24)	0.96	0.58
Age 7	Model 2	3,014	-0.12 (-0.16, -0.08)	-0.10 (-0.13, -0.06)	$1.6x10^{-08}$	67.6	0.11	-0.18 (-0.56,0.20)	-0.11(-0.34, 0.12)	0.35	0.92
	Model 3†	2,538	-0.09 (-0.14, -0.05)	-0.08 (-0.12, -0.04)	0.00003	46.8	0.10	-0.11 (-0.58,0.38)	-0.06(-0.36, 0.23)	0.68	0.92
FMI(kg/m2)	Model 1	3,014	-0.01 (-0.02, -0.001)	-0.04 (-0.07, -0.002)	0.04	100	0.02	-0.01 (-0.33, 0.32)	-0.004 (-0.21, 0.20)	0.97	0.81
Age 9	Model 2	3,014	-0.03 (-0.04, -0.02)	-0.09 (-0.13, -0.06)	$2.4x10^{-07}$	90.3	0.11	-0.18 (-0.51, 0.14)	-0.11(-0.32, 0.09)	0.28	0.76
	Model 3†	2,538	-0.02 (-0.03, -0.01)	-0.07 (-0.11, -0.03)	0.002	74.1	0.11	-0.07 (-0.47, 0.34)	-0.04(-0.28, 0.21)	0.75	0.76

† 476 individuals with missing data N=Number B=Beta coefficient CI = Confidence interval std = standardized
p = strength of evidence against the null hypothesis of no association
p(DWH) is the p-value of the Durbin form of the DWH test, which examines the difference between the estimates from linear regression and instrumental variable analysis
Model 1 adjusted for age and sex Model 2 adjusted for Model 1 and other thyroid hormone parameters
Model 3 adjusted for Model 2 and height, markers of social class and early life environment (home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score)

Similar results were also observed when assessing FT3 and adiposity data for individuals at age 15 years (**Tables 26 and 27**) with no evidence of difference in the regression estimates at ages 7 and 15 years (p=0.88).

Table 26 Associations between the weighted allelic BMI score for 32 SNPs and measures of adiposity at ages 7, 9 and 15 years.

Measures of adiposity	Per allele effects				
	β	95%CI	β (std)	95%CI	p*
BMI (age 7)	0.07	0.06, 0.09	0.04	0.03, 0.04	6.41×10^{-17}
FMI (age 9)	0.01	0.007, 0.01	0.04	0.04, 0.05	4.87×10^{-21}
Fat mass age 9 (kg)	0.20	0.16, 0.25	0.04	0.03, 0.05	3.71×10^{-20}
BMI (age 15)	0.16	0.12, 0.19	0.05	0.04, 0.16	1.16×10^{-16}
FMI (age 15)	0.01	0.008, 0.01	0.04	0.03, 0.05	1.06×10^{-10}
Fat mass age 15 (kg)	0.03	0.02, 0.04	0.04	0.03, 0.05	1.62×10^{-11}

*Calculated using the Wald test
B=Beta coefficient
CI = Confidence interval
p = strength of evidence against the null hypothesis of no association
std = standardized

Table 27 Associations between body mass index/fat mass index and FT3levels at age 15 as tested both by conventional epidemiological approaches and through the application of instrumental variable analysis using a 32-SNP weighted allelic score

Adiposity	FT3age 15	N	Linear Regression			Instrumental Variable Regression (Weighted Allelic Score with 32 SNPs)					
			β (95% CI)	B (std) (95% CI)	p-Value	*F*-Statistic	Partial *R*2	β (95% CI)	B(std) (95% CI)	p-Value	p-Value (DWH)
BMI (kg/m2)	Model 1	730	0.04 (-0.01, 0.09)	0.06 (-0.01, 0.12)	0.09	32.2	0.10	0.31 (0.05, 0.57)	0.42 (0.07, 0.77)	0.02	0.02
Age 15	Model 2		0.05 (-0.004, 0.10)	0.07(-0.001, 0.13)	0.05	32.4	0.11	0.35 (0.09, 0.60)	0.47 (0.12, 0.81)	0.008	0.01
	Model 3†		0.04 (-0.01, 0.09)	0.06 (-0.01, 0.12)	0.10	28.7	0.12	0.34 (0.07, 0.61)	0.46 (0.09, 0.83)	0.02	0.02
FMI (kg/m2)	Model 1	730	0.04 (-0.02, 0.10)	0.06 (-0.03, 0.14)	0.19	29.1	0.08	0.40 (0.07, 0.74)	0.54 (0.09, 1.00)	0.02	0.02
Age 15	Model 2		0.05 (-0.01, 0.11)	0.06 (-0.02, 0.14)	0.13	29.3	0.08	0.45 (0.11, 0.79)	0.60 (0.15, 1.06)	0.009	0.008
	Model 3†		0.04 (-0.02, 0.10)	0.05 (-0.03, 0.14)	0.24	27.1	0.10	0.43 (0.08, 0.78)	0.57 (0.11, 1.04)	0.02	0.01

†51 children with missing details
β=Beta coefficient
CI = Confidence interval
std = standardized
p = strength of evidence against the null hypothesis of no association
p(DWH) is the p-value of the Durbin form of the DWH test, which examines the difference between the estimates from linear regression and instrumental variable analysis

Model 1 adjusted for age and sex
Model 2 adjusted for Model 1 and other thyroid hormone parameters
Model 3 adjusted for Model 2 and markers of social class and early life environment (home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score)

4C.3.3 MR analysis using multiple independent instruments

Results of genetic analysis using rs1558902 in *FTO* (the individual SNP with the largest effect size on BMI) were compared with those of a weighted allelic score consisting of the remaining 31 genetic variants (**Tables 28 and 29)** and both revealed a positive association with individuals with a higher BMI having higher FT3 levels making pleiotropy unlikely. Again, no evidence of association was observed with FT_4 levels (**Tables 28 and 29**).

Table 28 Associations between body mass index/fat mass index and FT3 and FT4 levels at age 7 as tested both by conventional epidemiological approaches and through 31 SNPs (excluding FTO)

Adiposity	Model	N	Linear Regression			Instrumental Variable Regression (Weighted Allelic Score with 31 SNPs)					
			β (95% CI)	β (std) (95% CI)	*p*-Value	*F*-Statistic	Partial *R*2	β (95% CI)	B(std) (95% CI)a	p-Value	p-Value (DWH)
			31 SNPs (all SNPs excluding *FTO*)								
			FT3age 7								
BMI(kg/m2)	Model 1	3,014	0.05 (0.04, 0.06)	0.13 (0.10, 0.16)	$5.3x10^{-16}$	73.6	0.008	0.19 (0.04, 0.33)	0.30 (0.07, 0.53)	0.01	0.17
Age 7	Model 2	3,014	0.05 (0.04, 0.06)	0.16 (0.13, 0.19)	$6.6x10^{-21}$	75.1	0.10	0.19 (0.05, 0.32)	0.30 (0.08, 0.52)	0.007	0.21
	Model 3†	2,538	0.04 (0.03, 0.05)	0.12 (0.08, 0.16)	$3.0x10^{-13}$	57.9	0.07	0.20 (0.03, 0.38)	0.33 (0.04, 0.61)	0.02	0.14
FMI(kg/m2)†	Model 1	3,014	0.22 (0.17, 0.27)	0.17 (0.13, 0.20)	$1.5x10^{-22}$	98.1	0.04	0.16 (0.04, 0.29)	0.26 (0.06, 0.46)	0.01	0.45
Age 9	Model 2	3,014	0.23 (0.19, 0.27)	0.19 (0.16, 0.23)	$2.1x10^{-18}$	98.6	0.12	0.17 (0.05, 0.28)	0.27 (0.08, 0.46)	0.06	0.43
	Model 3†	2,538	0.17 (0.12, 0.22)	0.13 (0.09, 0.17)	$3.5x10^{-18}$	77.6	0.10	0.17 (0.03, 0.32)	0.28 (0.04, 0.51)	0.02	0.26
			FT4 age 7								
BMI(kg/m2)	Model 1	3,014	-0.07 (-0.10, -0.03)	-0.05 (-0.08, -0.02)	0.001	71.9	0.01	0.03 (-0.35, 0.41)	0.02 (-0.21, 0.25)	0.88	0.51
Age 7	Model 2	3,014	-0.12 (-0.16, -0.08)	-0.10 (-0.13, -0.06)	$1.6x10^{-08}$	69.9	0.11	-0.14 (-0.51, 0.23)	-0.09 (-0.31, 0.14)	0.46	0.90
	Model 3†	2,538	-0.09 (-0.14, -0.05)	-0.08 (-0.12, -0.04)	0.00003	48.7	0.10	-0.06 (-0.54 0.41)	-0.04 (-0.33, 0.25)	0.79	0.79
FMI(kg/m2)†	Model 1	3,014	-0.01 (-0.02, -0.001)	-0.04 (-0.07, -0.002)	0.04	105	0.01	-0.003 (-0.33, 0.32)	-0.01 (-0.20, 0.20)	0.99	0.70
Age 9	Model 2	3,014	-0.03 (-0.04, -0.02)	-0.09 (-0.13, -0.06)	$2.4x10^{-07}$	103	0.11	-0.16 (-0.49, 0.17)	-0.10 (-0.29, 0.10)	0.35	0.99
	Model 3†	2,538	-0.02 (-0.03, -0.01)	-0.07 (-0.11, -0.03)	0.002	74.2	0.10	-0.05 (-0.45, 0.35)	-0.03 (-0.27, 0.21)	0.80	0.70

† 476 individuals with missing data

B=Beta coefficient, CI = Confidence interval, std = Standardized, p = strength of evidence against the null hypothesis of no association

P (DWH) is the p-value of the Durbin form of the DWH test, which examines the difference between the estimates from linear regression and instrumental variable analysis Model 1 adjusted for age and sex, Model 2 adjusted for Model 1 and other thyroid hormone parameters, Model 3 adjusted for Model 2 and height markers of social class and early life environment (home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score)

Table 29 Associations between body mass index/fat mass index and FT3 and FT4 levels at age 7 as tested both by conventional epidemiological approaches and through the FTO SNP

Adiposity	Model	N	Linear Regression			Instrumental Variable Regression FTO					
			β (95% CI)	β (std) (95% CI)	*p*-Value	*F*-Statistic	Partial *R*2	β (95% CI)	β (std) (95% CI)	p-Value	p-Value (DWH)
			***FTO* SNP alone**								
			FT3age 7								
BMI(kg/m2)	Model 1	3,014	0.05 (0.04, 0.06)	0.13 (0.10, 0.16)	$5.3x10^{-16}$	10.6	0.64	0.61 (0.12, 1.09)	0.97 (0.20, 1.75)	0.01	0.006
Age 7	Model 2	3,014	0.05 (0.04, 0.06)	0.16 (0.13, 0.19)	$6.6x10^{-21}$	11.8	0.61	0.63 (0.15, 1.11)	1.02 (0.25, 21.80)	0.01	0.003
	Model 3†	2,538	0.04 (0.03, 0.05)	0.12 (0.08, 0.16)	$3.0x10^{-13}$	9.52	0.67	0.72 (0.02, 1.43)	1.17 (0.03, 2.30)	0.04	0.007
FMI(kg/m2)	Model 1	3,014	0.22 (0.17, 0.27)	0.17 (0.13, 0.20)	$1.5x10^{-22}$	9.87	0.31	0.48 (0.14,0.82)	0.78 (0.23, 1.32)	0.006	0.01
Age 9	Model 2	3,014	0.23 (0.19, 0.27)	0.19 (0.16, 0.23)	$2.1x10^{-78}$	15.9	0.26	0.50 (0.17,0.84)	0.82 (0.28, 1.36)	0.003	0.008
	Model 3†	2,538	0.17 (0.12, 0.22)	0.13 (0.09, 0.17)	$3.5x10^{-18}$	14.6	0.24	0.52 (0.10, 0.94)	0.84 (0.16, 1.52)	0.008	0.02
			FT4 age 7								
BMI(kg/m2)	Model 1	3,014	-0.07 (-0.10, -0.03)	-0.05 (-0.08, -0.02)	0.001	10.6	0.01	-0.21 (-1.20, 0.79)	-0.13 (-0.73, 0.47)	0.68	0.82
Age 7	Model 2	3,014	-0.12 (-0.16, -0.08)	-0.10 (-0.13, -0.06)	$1.6x10^{-08}$	7.95	0.02	-0.77 (-1.96, 0.41)	-0.47 (-1.18, 0.25)	0.20	0.28
	Model 3†	2,538	-0.09 (-0.14, -0.05)	-0.08 (-0.12, -0.04)	0.00003	5.27	0.01	-0.68 (-2.16, 0.83)	-0.41 (-1.33, 0.50)	0.37	0.45
FMI(kg/m2)	Model 1	3,014	-0.01 (-0.02, -0.001)	-0.04 (-0.07, -0.002)	0.04	18.1	0.01	-0.20 (-1.00, 0.60)	-0.12 (-0.61, 0.31	0.63	0.74
Age 9	Model 2	3,014	-0.03 (-0.04, -0.02)	-0.09 (-0.13, -0.06)	$2.4x10^{-07}$	14.2	0.03	-0.65 (-1.56, 0.26)	-0.39 (-0.94, 0.16)	0.16	0.27
	Model 3†	2,538	-0.02 (-0.03, -0.01)	-0.07 (-0.11, -0.03)	0.002	11.2	0.07	-0.50 (-1.55, 0.56)	-0.30 (-0.93, 0.35)	0.36	0.49

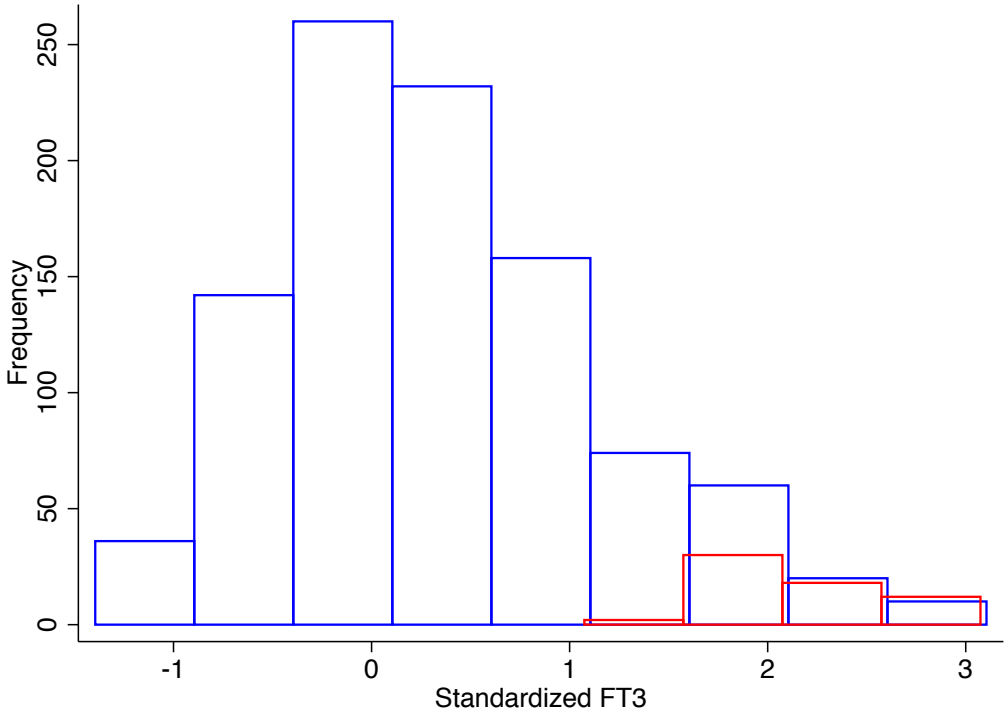
† 476 individuals with missing data

β=Beta coefficient, CI = Confidence interval, std = standardized, p = strength of evidence against the null hypothesis of no association

P (DWH) is the p-value of the Durbin form of the DWH test, which examines the difference between the estimates from linear regression and instrumental variable analysis Model 1 adjusted for age and sex, Model 2 adjusted for Model 1 and other thyroid hormone parameters, Model 3 adjusted for Model 2 and height markers of social class and early life environment (home ownership, maternal age at birth of child, maternal highest educational qualification, maternal smoking in pregnancy, family adversity index and parents and home score)

The instrumented effect for *FTO* on FT3 showed some difference to observational estimates, especially for both BMI and FMI, where the instrumental variable analysis produced larger effect estimates than the observational analysis (DWH p=≤0.01). There was also some evidence that the instrumented effects of BMI on FT3 were higher for *FTO* than the other 31 SNPs. An additional analysis identified that independent pairs of variants from the 32 SNPs have instrumental variable effects that are normally distributed (**Figure 12**). However pairs of variants which included *FTO* are at the upper end of this distribution, indicating that although variation in *FTO* produces a substantially higher impact on FT3than the average instrumental variable effect it is not an outlier.

Figure 12 Distribution of FT3 level for pair combinations of the 32 SNPs in instrumental variable regressions



Blue indicates the distribution of FT3 levels for all pair combinations of the 32 SNPs; red indicates the distribution of FT3 for combinations of the 32 SNPs where one SNP was the *FTO* (rs1558902) variant. Variation in *FTO* clearly has the most substantial effect on body composition and was the first obesity marker to be identified by GWAS [231]. In keeping with this *FTO* had by far the most substantial effect of any SNP in this analysis.

4C.4 DISCUSSION

Analysis using my genetic instruments revealed that individuals with a genetically higher BMI/fat mass had higher FT3 levels in keeping with observational estimates. In contrast, there was no evidence of association between individuals with a genetically higher BMI/fat mass

and FT4 levels. Interestingly the observational positive association between BMI and FT3 appears to be substantially weaker at age 15, however IV analysis effect estimates were more comparable. Furthermore the effect also still appears to be present in adults as previous studies also identified a positive association between FT3 and BMI in healthy euthyroid men aged between 25-45 years [207, 209]. Therefore repeating this MR analysis in adults would be particularly informative as would further studies comparing the biology of fat in children and adults in this regard.

Taken together, our data suggests that higher levels of BMI and adiposity cause an increase in serum FT3 levels but do not appear to influence FT4 levels. This would explain the paradoxical opposing relationship of FT3 (positive) and FT4 (negative) with BMI and fat mass in observational studies. It is also notable that our overall genetic effect estimates of fat mass on FT3 were substantially higher than the observational analysis. This may indicate that the higher FT3 generated from higher fat mass has a negative effect on fat mass, but this is a much weaker overall effect than the positive effect of fat mass on FT3. This is in keeping with a recent study which identified obese children have higher FT3 levels [232].

Whilst our genetic analyses indicate the nature of the relationship between fat mass and FT3 the mechanism of action for increased fat mass increasing FT3 remains unclear. A simplistic assumption could be that increased fat mass results in increased generation of FT3 from FT_4 and

this increased FT3 production in fat leads to increased FT3 in serum. However, this would require increased DIO2 conversion of FT4 to FT3 in fat and this enzyme is expressed in brown but is not substantially expressed in white adipose tissue [233].

Alternatively, increased fat mass may result in other alterations in the HPT axis as longitudinal analysis in ALSPAC has indicated that FT3 is less influenced by TSH levels than FT4 and has greater intra-individual variation (**Chapter 4A**). Alternatively observed changes in FT3 may relate in part to excess carbohydrate in the diet of obese individuals [234]. In addition, our analysis showed children have higher FT3 levels than adults with almost 25% of children having a FT3 above the adult reference-range (**Chapter 4A**). This implies that other factors influence FT3 levels in young children although fat mass may still have a substantial role. Further insight may be available from whole genome sequence analysis of body mass and thyroid function, however they are unlikely to substantially increase the variance explained at present [217].

Although the MR method is more resistant to reverse causation and confounding than traditional observational epidemiological studies, there are limitations to this approach. Polygenic score analyses for TSH and FT4, have not revealed a common genetic determination with metabolic and anthropometric measures [217] although FT3 has not been studied it is unlikely it shares a common genetic architecture (pleiotropy) to fat mass. Our use of 32 independent alleles in determining the gene score,

substantially reduced the risk of shared pleiotropy and linkage-disequilibrium-induced confounding [230]. Furthermore, our use of two separate genetic instruments substantially reduced the risk of pleiotropy. Another limitation was that we did not have data on thyroid function, body composition or genetic architecture in a substantial number of children in the ALSPAC cohort. However, this would only lead to bias if the causal effects of higher fat mass increasing FT3 levels are different in the children not studied in this study. Although we cannot fully exclude this there was no substantial difference in our models after adjustment for relevant socio-economic confounders. Furthermore positive associations between adiposity and FT3 were also observed when using different instrument combinations, suggesting that there is not a systematic and biasing effect of pleiotropy in this case.

With regard to measurement of free thyroid hormones, biases have been reported [235], whilst measured levels of free thyroid hormones in our study may not be entirely independent from thyroid binding globulin levels, the striking difference observed in the associations between BMI allelic score and FT3 and FT4 makes a substantial impact from thyroid binding globulin in our genetic analysis unlikely. Previous analyses have also identified the association between thyroid hormone parameters and body composition was largely independent of thyroid binding globulin [155]. Potentially FT3 levels may be reduced in children with recent illness before blood sampling but this would have likely biased our genetic associations to the null.

More recent data[236] on the genetic architecture of body composition than that used in our analyses[216] is now available. This new data [236] explains more of the variation in BMI and identified over 50 novel SNPs. Re-analysing using these SNPs in a future study will confirm our findings and may allow greater insight into the pathways through which fat mass increases FT3.

As well as providing insight into the regulation of FT3 in children, our findings are potentially clinically relevant. Childhood obesity is common and rising [237] and increased FT3 levels arising from increasing fat mass may have long-term consequences, particularly at the population-level as even modest variation in thyroid status within the population reference-range has adverse phenotypic effects (**Chapter 4B)**.

In conclusion, our analysis in this chapter has indicated that BMI and adiposity causally increase FT3 levels in children. More research is required to identify the causal mechanisms for this and the consequences of childhood obesity on this relationship.

Chapter 5 Thyroid hormone replacement in the UK for primary hypothyroidism.

As we have already seen in this thesis, modest variation in thyroid status can have a substantial impact on key health outcomes. Given hypothyroidism is common and levothyroxine is being widely and increasingly used patterns and trends in levothyroxine prescribing may have important implications for adult health. There has been concern that individuals are being started on levothyroxine for modest elevations in TSH and some evidence that many individuals may end up being over-treated. This could result in patients moving from borderline low thyroid function to high thyroid function, which could substantially modify the risks of adverse outcomes moving from cardiovascular and metabolic risk factors to adverse bone outcomes. In this chapter, I utilized primary care data of over 50,000 people started on levothyroxine for primary hypothyroidism and explored temporal trends in management.

5.1 INTRODUCTION

Primary hypothyroidism is one of the commonest chronic conditions with a prevalence between 1-2% [10, 34] and is largely managed in primary care [238, 239]. Recently it has been observed that levothyroxine prescriptions in England and Wales have increased substantially over recent years, rising from 17.1 million in 2006 to 23.4 million in 2010 [240], up from only 7 million prescriptions in 1998 [241, 242].

Several factors are likely to have contributed to this rise. A proportion may be attributed to a fall in the average duration of prescriptions from 60 to 45 days [242]. What is likely to have had a bigger impact is that thyroid function testing has also increased substantially [52, 243] and data from the USA has revealed that in any year 18-25% of individuals have their thyroid function tested [239, 243, 244]. This has therefore likely resulted in increased case-finding of hypothyroidism. However, an additional factor might be a lowering of the TSH threshold at which levothyroxine is initiated. This practice would be important to identify, as this might be associated with more marginal benefits and increased relative risk of patient harm. As indicated earlier in this thesis even variation within the population reference range might have substantial effects on key health outcomes (**Chapter 3).** Over-treatment is associated with an increased risk of both fractures [245] and atrial fibrillation [246]. As indicated earlier in this thesis, analyses from population-based cohorts studying the effects of variation in thyroid status within the normal population range might suggest that overtreatment of individuals with marginally elevated TSH levels might result in net harm.

In this chapter I used a large UK population-based database to examine trends in TSH levels pre-and post levothyroxine initiation since 2001 up to 2009, to assess the potential for adverse outcomes from current practice in the general adult population. This will provide key data in determining how much our practice of managing hypothyroidism modifies

risk factors for adverse adult health outcomes which we observed in **Chapter 3.**

5.2 METHODS

5.2.1 Study populations

Clinical data and dates of levothyroxine prescriptions and TSH levels were extracted on primary care patients from the GPRD (now called the Clinical Practice Research Datalink www.CPRD.com). This has been described already in **Chapter 2**. To summarize The CPRD is the largest computerized database of anonymized medical records from primary care linked with other healthcare data. It is well validated for research on clinical diagnoses [125, 126], drug exposure and patient safety [122-124]. At the time of this study the GPRD contained computerized medical records of over 5,000,000 people from 508 primary care practices throughout the UK. The clinical data is entered and stored as codes. This enabled us to access large amounts of clinical data enabling us to explore both biochemical TSH levels before and after levothyroxine initiation, and also investigate symptoms that led to thyroid function testing and investigate as well as relevant comorbidities. Whilst this approach has key advantages, a notable limitation is it relies on data being entered by primary care physicians accurately and whilst entered data has a high positive predictive value, it may not be sensitive as diagnoses may not be entered.

Patients were included in our dataset if they were aged between 18-99 years at their first-ever prescription of levothyroxine which occurred between 01/01/2001-30/10/2009. For inclusion in this analysis patients also needed at least 12 months of up-to-standard (data that met GPRD quality standards) follow-up prior to their index levothyroxine. This required patients to have been at the same practice, with regular monitoring and attendance for repeat prescriptions. Patients with a prescription record at any time of amiodarone, carbimazole, propylthiouracil, lithium, interferon, thalidomide or sunitinib were excluded; as were patients diagnosed with or treated for hyperthyroidism prior to their index levothyroxine as evidenced by medical codes (related to ICD-10 codes) and referral codes in the GPRD relating to Graves' disease, thyrotoxicosis, hyperthyroidism, toxic multi-nodular goiter, toxic nodule, thyroiditis, thyroidectomy and radio-iodine. Patients with a documented diagnosis of thyroid cancer or a diagnosis of pituitary disease/pituitary surgery were excluded. Individuals with levothyroxine initiation related to pregnancy were also excluded. To identify levothyroxine prescriptions related to pregnancy, 987 medical codes were used to identify prescriptions motivated by pregnancy or pre-conception planning. These pregnancy codes were provided with assistance from Caroline Minassian (London School of Hygiene and Tropical Medicine). These codes included antenatal appointments, confirmation of pregnancy tests and delivery outcomes.

A pregnancy medical code was regarded as relevant to the prescription of levothyroxine if it occurred up to 365 days before or up to 40 weeks after levothyroxine initiation.

5.2.2 Identification of TSH and FT_4 results generating index levothyroxine prescription

We studied incident (first) levothyroxine prescriptions. A TSH or FT4 level was deemed relevant if it occurred within the 90 days before levothyroxine initiation. If more than 1 result was available, then the result closest to the date of levothyroxine initiation was used. Prescribing rates were calculated using baseline GPRD denominator data and were adjusted after removing from the denominator the person-time of individuals prescribed levothyroxine after 2001 (from the date of their levothyroxine prescription until the end of the study period or their exit from the GPRD). Excluded individuals (e.g., those prescribed levothyroxine in pregnancy) were also removed from the person-years at risk.

34,808 individuals had an interpretable FT_4 level available (FT_4 level with data of the normal assay range also available) at their index prescription.

5.2.3 Identification of factors potentially relevant to prescribing levothyroxine at the time of initiation of treatment

Medical codes entered within 60 days before the relevant thyroid function test were studied for each patient. Codes regarding symptoms, examination findings, diagnoses, clinic appointments, and investigations were grouped into categories specified *a priori*. For example, the atrial

fibrillation or tachycardia category had several medical codes, including atrial fibrillation, AF, and paroxysmal AF pertaining to it. Individuals could be assigned to more than 1 category but would only be counted once within a category.

5.2.4 TSH levels post-levothyroxine

Using the date of index levothyroxine as time zero, the TSH levels post-levothyroxine therapy were studied for up to 5 years. Time bands were split into 6-month intervals. Individuals could only appear once in each time-band. If 2 or more TSH values were available for a patient in the same 6-month period, the later TSH level was used. We studied TSH values 30-36 months and 54-60 months after levothyroxine initiation. TSH levels below 0.5mU/l were regarded as low and values below 0.1mU/l were regarded as suppressed in keeping with previous regional UK studies [52, 246]. FT4 levels between 10-20 pmol/l were classified as within the normal range.

5.2.5 Statistical methods for identifying differences in prescribing

Median TSH levels at levothyroxine initiation were calculated by year between 2001-2009. Logistic regression was undertaken to assess the odds ratio of having a first levothyroxine prescription with TSH levels less than 10mU/l using the odds of being prescribed levothyroxine with a TSH lower than 10mU/l in 2001 as a baseline, with analyses adjusted for age at levothyroxine initiation, sex and clinical characteristics.

Univariable logistic regression was also used to estimate the odds of developing a suppressed TSH at 5 years post levothyroxine for sex, age, year, TSH at index levothyroxine prescription and key clinical characteristics prior to levothyroxine therapy. Multivariable logistic regression was then undertaken adjusting for sex, age, year, and TSH at index levothyroxine prescription.

5.2.6 Regulatory approval

Access to the GPRD dataset was obtained via the Medical Research Council license. The study protocol was approved by the Independent Scientific Advisory Group of the UK Medicines and Healthcare products Regulatory Agency.

5.3 RESULTS

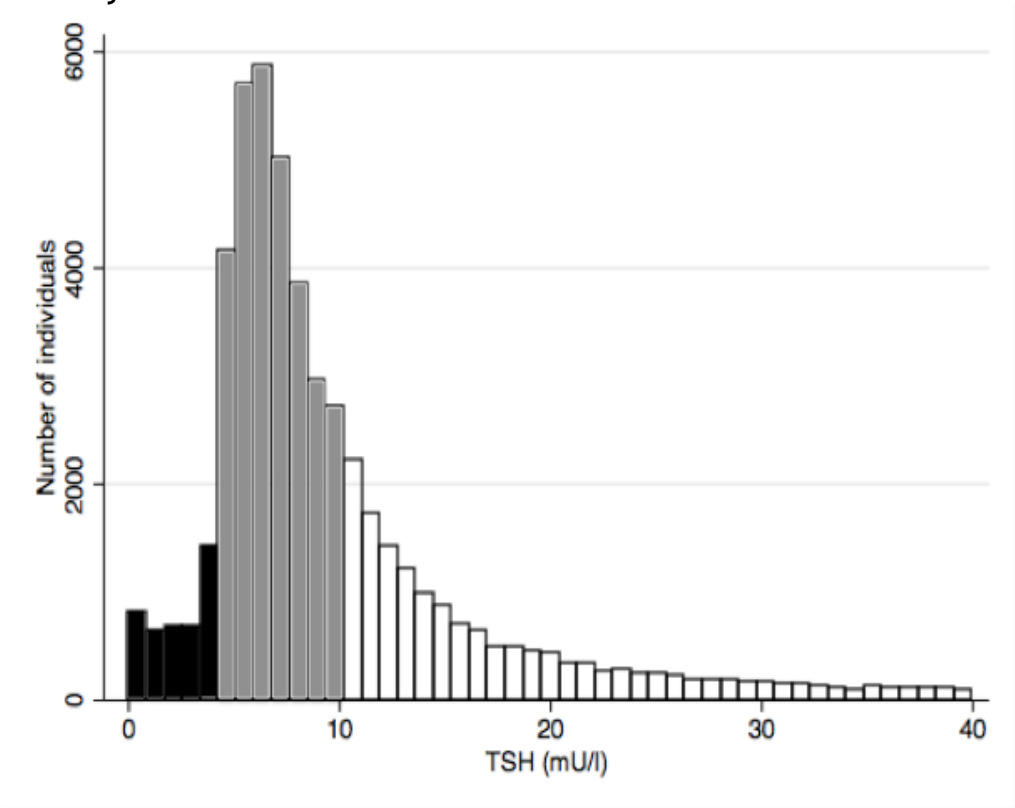
5.3.1 Characteristics of individuals prescribed levothyroxine

We identified 52,298 individuals matching our inclusion criteria who had a levothyroxine prescription within 90 days after a documented TSH level. The median age at index levothyroxine was 59 years (IQR 47-72) with a Male: Female ratio of 1:3.74.

5.3.2 Prescribing patterns in initiating levothyroxine therapy

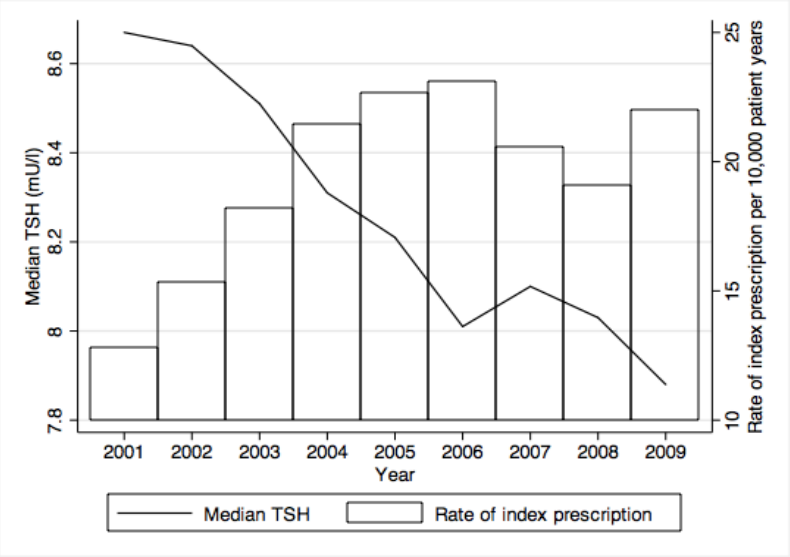
Overall the median TSH prior to index levothyroxine between 2001-2009 was 8.2mU/l (IQR 5.9–13.9) (**Figure 13**). The annual median TSH level fell over the study period, from 8.7mU/l to 7.9mU/l (**Figure 14**).

Figure 13 TSH Levels at the Time of the Index Prescription of Levothyroxine



Dark bars represent TSH levels < 4.0 mU/l, medium bars TSH levels 4.0-10.0 mU/l and light bars represent TSH levels > 10.0 mU/l. These cutoffs were used as 4.0mU/l is often the quoted upper limit of the normal range and TSH levels >10 are a definite indication for treatment.

Figure 14 Median Thyrotropin Levels at the Time of the Index Prescription of Levothyroxine and Rate of Index Prescriptions by Year



This fall reflected a reduction in individuals with an initial TSH level greater than 10mU/l (42.1% to 35.9%) and a rise in those treated for a TSH in the range 4-10mU/l (49.8% to 58.1%) (**Table 30)**. The odds ratio of having an index levothyroxine prescription with a TSH level less than 10mU/l in 2009 compared to 2001, adjusting for age at prescription, sex, presence of diabetes/hypertension/raised lipids, and presenting symptom was 1.30 (95%CI 1.19, 1.42; p=<0.001). Free thyroxine (FT_4) levels were available in 34,808 (66.6%) subjects at index prescription. The odds of starting levothyroxine with a TSH of <10mU/l at the end of the study in the subgroup of subjects with a FT_4 in the reference range was slightly lower OR=1.17 (95%CI 1.00, 1.36; p=0.05) than in the whole cohort analysis (OR=1.30).

Table 30 TSH levels prior to index levothyroxine prescription by year and the odds of an index prescription of levothyroxine arising from a TSH less than 10mU/l by year, using prescribing data of levothyroxine in 2001 as baseline

Year	% TSH < 4.0mU/l	% TSH 4 -10mU/l	% TSH > 10 mU/l	Odds Ratio	Model 1# 95%CI	p value*	Odds Ratio	Model 2# 95%CI	p value*	Odds Ratio	Model 3# 95%CI	p value*
2001	8.08	49.8	42.1	1			1			1		
2002	5.57	53.1	41.3	1.03	0.94 - 1.12	0.49	1.02	0.94 - 1.12	0.59	1.02	0.93 - 1.11	0.68
2003	5.51	53.3	41.2	1.04	0.95- 1.12	0.41	1.04	0.95 - 1.13	0.45	1.03	0.94 - 1.12	0.53
2004	6.63	54.3	39.1	1.14	1.04 - 1.23	0.003	1.14	1.05 - 1.24	0.002	1.13	1.04 - 1.22	0.005
2005	5.44	56.0	38.5	1.16	1.04 - 1.25	<0.001	1.17	1.08 - 1.27	<0.001	1.14	1.05 - 1.24	0.001
2006	5.84	57.4	36.7	1.27	1.15- 1.35	<0.001	1.27	1.17 - 1.38	<0.001	1.24	1.14 - 1.34	<0.001
2007	5.22	57.3	37.4	1.22	1.11 - 1.32	<0.001	1.23	1.13 - 1.34	<0.001	1.19	1.10 - 1.31	<0.001
2008	6.67	55.8	37.5	1.18	1.11 - 1.32	<0.001	1.24	1.14 - 1.35	<0.001	1.20	1.10 - 1.31	<0.001
2009	6.28	58.1	35.6	1.32	1.20 - 1.43	<0.001	1.34	1.23 - 1.46	<0.001	1.30	1.19 - 1.42	<0.001

52,298 individuals in model * Calculated using the Wald test
Model 1 Crude, Model 2 Adjusted for age at levothyroxine initiation, and sex, Model 3 Adjusted for age at levothyroxine initiation, sex diabetes prior to levothyroxine initiation, hypertension or raised lipid levels prior to levothyroxine initiation and presenting symptom.

Between 2001-2006 there was a 1.81 fold increase in the rate of index levothyroxine prescriptions (**Table 31**) After this time the rate of new prescriptions did not substantially change despite a continuing decline in the median TSH at index levothyroxine (**Figure 14**). Age-standardized rates comparing 2001 prescribing to 2006 prescribing revealed that there was still a 1.79 fold increase in the rate of index levothyroxine prescriptions after the change in age in the dataset was taken into account. Age-stratified rates are shown in **Table 32**. Levothyroxine prescriptions were usually continued long-term: 38,939 of the 43,057 individuals (90.4%) still in the GPRD at the end of the study received a repeat levothyroxine prescription during 2009.

Table 31 GPRD Population and prescribing patterns by year

Year	Total Population in GPRD aged 18 years +	Person years	Number of prescriptions	Rate per 10,000 person years	Proportion of GPRD started on Levothyroxine	Adjusted rate* per 10,000 years
2001	3085170	2792322	3576	128.1	1.16×10^{-3}	129.5
2002	3189619	2925037	4488	153.4	1.41×10^{-03}	155.2
2003	3225512	2974217	5413	182.0	1.68×10^{-03}	184.4
2004	3291684	3038516	6514	214.4	1.98×10^{-03}	217.4
2005	3341246	3095391	7005	226.3	2.10×10^{-03}	230.0
2006	3415872	3131345	7226	230.8	2.12×10^{-03}	234.7
2007	3451212	3165081	6512	205.7	1.89×10^{-03}	209.5
2008	3430267	3140549	5985	190.6	1.74×10^{-03}	194.3
2009	3400030	2518483	5539	220.0	1.96×10^{-03}	225.7

*Rate adjusted for individuals no longer at risk of being prescribed levothyroxine for the first time, as a result of levothyroxine prescription, exclusion due to pregnancy, medication and pituitary disease from our dataset.

Table 32 Age stratified rates of levothyroxine prescription by year

Year / Age-group	2001		2002		2003		2004		2005		2006		2007		2008		2009	
	Prop	R	Prop	R	Prop	R	Prop	R	Prop	R	Prop	R	Prop	R	Prop	R	Prop	R
20-30	1.89	2.23	2.46	2.87	3.41	3.93	3.57	4.12	4.13	4.76	4.37	5.08	4.00	4.63	3.93	4.58	4.53	5.50
30-40	5.06	5.69	6.41	7.10	7.28	8.03	8.61	9.50	9.36	10.3	10.2	11.3	9.75	10.8	8.65	9.68	9.42	10.8
40-50	10.5	11.4	11.6	12.4	13.6	14.4	17.2	18.3	17.5	18.5	18.2	19.5	16.9	18.0	15.5	16.6	17.9	19.7
50-60	16.2	17.3	18.7	19.7	21.9	23.0	25.8	27.0	28.4	29.7	28.1	29.6	26.5	28.0	23.2	24.5	26.9	29.2
60-70	20.6	22.0	22.8	24.0	27.4	28.7	32.6	34.2	33.6	35.0	34.1	35.9	28.5	30.1	27.5	28.9	29.7	32.1
70-80	20.9	22.5	28.4	30.1	34.8	36.9	38.9	40.9	41.7	43.6	40.3	42.7	35.1	37.2	31.6	33.4	35.0	38.0
80-90	23.5	26.3	30.1	33.4	35.7	39.5	41.8	46.0	42.3	46.2	46.1	50.8	34.6	38.2	34.3	37.9	38.8	43.9
90-100	18.2	22.0	30.7	37.5	36.3	43.8	39.8	48.2	51.5	61.8	41.0	49.5	38.4	46.6	42.1	51.2	40.9	50.7
Overall	11.6	12.8	14.1	15.3	16.7	18.2	19.7	21.4	21.0	22.6	21.1	23.0	18.8	20.5	17.4	19.0	19.5	22.0

Prop = Proportion of individuals in GPRD ($x10^{-5}$) R =Rate per 10,000 patient years

The rate of index levothyroxine prescriptions also increased steadily with age (**Table 32**). As a proportion, of the at-risk population, the greatest number of new prescriptions was among those aged between 55-60 years. In males the median TSH at index prescription over the study period was higher than females (p=<0.001) 8.90mU/l (IQR 6.21–16.2) versus 8.05mU/l (IQR 5.84-13.4) in females. There was also a surprising dip in the rate of levothyroxine prescribing in 2007 and 2008 followed by a recovery, although there was no obvious change in guideline or practice around this time.

5.3.3 Clinical data in subjects prescribed levothyroxine

The symptoms and signs recorded in the 60-day period prior to initiating levothyroxine are shown in **Table 33**. The commonest symptoms were tiredness (19.3%), weight gain/obesity (14.0%) and depression (5.8%). Individuals with recorded sleep apnea (23.1mU/l), peri-orbital oedema (32.7mU/l) and Addisons (21.1 mU/l), had median TSH levels substantially greater than 10mU/l consistent with the presence of more profound hypothyroidism.

Table 33 Relevant symptoms, signs and diagnoses prior to initiation of levothyroxine

Clinical	Number of Individuals	Median TSH	(IQR)
Symptom			
Cold intolerance*	43	7.05	(5.30 – 14.2)
Loss of taste	12	7.22	(4.50 - 10.4)
Loss of libido	51	7.36	(5.03 – 13.5)
Depression	1462	7.60	(5.55 – 13.2)
Tired*	4839	7.74	(5.50 - 14.5)
Neck Pain	412	7.80	(5.59 – 12.3)
General aches and pains	625	7.81	(5.80 – 14.9)
Tremor	125	7.82	(5.88 – 11.2)
Sore tongue	158	7.86	(6.15 – 13.6)
Itch	410	7.87	(5.6 – 12.3)
Heat intolerance	314	7.96	(5.56 -13.3)
Disturbed Sleep	487	8.03	(5.87 – 15.0)
Rash	589	8.12	(5.81 – 15.8)
Menopause symptoms	546	8.22	(5.82 - 14.9)
Weight gain/obesity	3517	8.30	(5.80 – 16.2)
Feeling dizzy/faint	1201	8.31	(5.94 - 15.3)
Increasing falls	265	8.41	(6.20 – 14.4)
Weight loss	277	8.50	(5.87 – 13.8)
Constipation*	550	8.60	(5.98 – 14.8)
Palpitations	568	8.60	(6.43 – 14.6)
Hoarse Voice*	157	8.70	(5.83 – 40)
Menstrual irregularity*	1333	8.81	(6.02 – 15.9)
General malaise	367	9.00	(5.66 – 17.6)
Increasing frailty	19	9.08	(4.65 – 19.9)
Fullness/constriction in neck	134	9.96	(6.40 – 18.8)
Examination Findings			
Thyroid nodule	30	6.40	(2.51 – 11.3)
Dry skin*	273	7.80	(5.71 – 17.2)
Alopecia/hair loss	487	8.21	(5.78 – 13.2)
Goitre*	531	8.28	(5.7 - 24.9)
Atrial fibrillation/tachycardia	273	8.30	(6.08 – 13.5)
Peripheral oedema	1363	8.40	(6.10 - 16.3)
Bradycardia*	40	8.50	(5.38 – 17.9)
Vitiligo	34	10.5	(6.09 – 20.0)
Myxoedema*	202	11.3	(6.0 - 27.0)
Gynacomastia	4	12.0	(5.12 – 20.6)
Peri-orbital oedema	20	32.7	(6.96 – 95.0)
Screening			
Mental health review	246	7.03	(5.36 – 12.1)
Diabetes review	1415	7.31	(5.59 – 10.7)
General screening	1395	8.02	(5.45 – 14.81)
Geriatric Screen	309	8.03	(6.32 – 12.8)
Following other diagnosis			
IGT/IFG	10	5.77	(4.79 – 8.86)
PCOS	19	5.86	(4.15 – 9.20)
Dementia	103	7.40	(5.53 – 12.7)
Pernicious anaemia	62	7.77	(6.20 – 10.7)
High lipids*	680	7.80	(5.63 – 13.7)
Stroke	64	7.80	(6.02 - 10.9)
Type 2 DM	367	7.85	(6.00 – 14.0)
Coeliac	11	7.94	(6.75 – 14.0)
Carpal Tunnel Syndrome*	243	7.97	(5.57 – 14.6)
IHD	520	8.00	(6.14 – 13.1)
Macrocytosis	68	8.14	(5.85 – 10.9)
Infertility	212	8.28	(5.8 – 14.4)
Hyponatremia	36	8.98	(5.85 - 11.9)
Type 1 DM	19	9.70	(7.57 - 12.8)
Sjogrens	7	10.5	(5.26 – 10.9)
Addisons	6	21.1	(9.34 – 38.4)
Sleep apnea*	9	23.1	(8.39 – 28.8)

Summary for Table 33 on previous page
TSH – Thyroid stimulating hormone, IQR – Inter-quartile range IHD – Ischaemic heart disease, DM – Diabetes Mellitus, IGT Impaired glucose tolerance, IFG – Impaired fasting glucose, PCOS – Polycystic ovarian syndrome Total Number 27,519 from 25,067 individuals (47.9%) in the dataset *7,410 individuals only had these "classic" signs and symptoms of hypothyroidism

5.3.4 Number of thyroid function tests before index levothyroxine

Data were available on individuals from their inclusion in GPRD and was therefore available for several years prior to 2001. The median number of thyroid function tests before index levothyroxine was 2 (IQR 1-3). 58.2% of individuals were started on levothyroxine who had never had a documented TSH level greater than 10mU/l and 34.6% of individuals who were prescribed levothyroxine with a TSH between 4-10mU/l only had one value above 4.0mU/l (**Table 34**).

Table 34 Relative percentages of the number thyroid function tests performed prior to initiation of levothyroxine by prescription threshold

TSH level at index prescription of levothyroxine	Percentage of thyroid function tests performed prior to initiation of levothyroxine (%)					
	1	2	>2	1*	2*	>2*
4 - 10 mU/l	24.8	26.3	48.9	34.6	30.8	34.7
> 10 mU/l	47.7	23.5	17.8	65.3	23.0	11.7

* TSH values less than 4 mU/l excluded

34,808 individuals had an interpretable FT_4 level available (FT_4 level with data of the normal assay range also available) at their index prescription. Comparing individuals with FT_4 data available to the rest of our dataset, there was no difference in sex (p=0.62) or age group (p=0.14), however TSH levels were lower at index prescription in those with FT_4 available (p=0.01). In individuals with FT_4 data available 38.8% had a TSH >10mU/l and 50.3 % had either a TSH >10mU/l or a low FT_4.

In the 34,808 individuals with a FT4 available 10, 939 (31.4%) had levothyroxine prescribed with a TSH level <10mU/l and a normal FT4 despite no previous cardiovascular risk factors or classic hypothyroid symptoms. In addition individuals starting levothyroxine with a TSH in the range 4-10mU/l and a normal FT4 rather than a low FT4 were more likely to be older and have cardio-vascular risk-factors, but not to have tiredness obesity or depression at baseline (**Table 35**). Individuals

prescribed levothyroxine with a TSH between 4-10mU/l rather than a TSH >10mU/l were more likely to be female, aged over 70, prescribed levothyroxine after 2004, or have cardiovascular risk factors, with trends also observed for depression/tiredness (**Table 36**).

Table 35 Odds of having levothyroxine initiated with a normal FT4 compared to a low FT4 in individuals with a TSH between 4-10 mU/l

Characteristic	CRUDE			ADJUSTED		
	Odds ratio	(95%CI)	p value*	Odds ratio#	(95%CI)#	p value#*
Sex						
Male	1			1		
Female	0.97	0.88- 1.06	0.50	1.01	0.91 – 1.10	0.92
Age group						
18 – 45	1			1		
45 – 70	0.97	0.88 – 1.07	<0.55	0.92	0.87 – 1.07	0.13
70 – 99	1.18	1.06 – 1.32	<0.003	1.05	0.93 – 1.18	0.43
Year of index prescription						
2001 -2003	1			1		
2004 – 2006	1.14	1.03 – 1.26	0.01	1.13	1.02 – 1.25	0.02
2007 – 2009	1.04	0.94 – 1.14	0.47	1.03	0.93 – 1.14	0.54
Classic hypothyroid sx						
No	1			1		
Yes	1.01	0.90 – 1.14	0.83	1.03	0.91, 1.16	0.63
Presence of AF						
No	1			1		
Yes	1.70	1.50 - 1.93	<0.001	1.43	1.16 – 1.74	0.001
Raised blood pressure/lipids						
No	1			1		
Yes	1.36	1.28 – 1.42	<0.001	1.19	1.09 – 1.30	<0.001
Presence of Diabetes						
No	1			1		
Yes	1.27	1.10 – 1.45	0.001	1.18	1.03 – 1.35	0.02
Clinical reasons for TSH measurement						
Depression	0.87	0.69 – 1.09	0.23	0.89	0.71 – 1.12	0.34
Tired	1.27	0.89 – 1.81	0.19	1.34	0.92 – 1.87	0.14
Weight gain/obesity	0.96	0.64 – 1.44	0.84	0.99	0.66 – 1.49	0.99
Peripheral Oedema	1.07	0.83 – 1.39	0.61	0.99	0.76 – 1.28	0.94
Menstrual irregularities	0.64	0.54 - 0.81	<0.001	0.67	0.54 – 0.85	0.001
Diabetes review	1.04	0.82 – 1.32	0.73	0.85	0.65 – 1.11	0.23
General Screening	0.84	0.65 – 1.08	0.17	0.82	0.63 – 1.05	0.12

19,801 individuals in model # Adjusted for age at prescription, year at prescription, sex, cardiovascular risk factors and presence of classic symptoms

Table 36 Odds of having levothyroxine initiated with a TSH between 4-10 mU/l compared to a TSH greater than 10

Characteristic	CRUDE			ADJUSTED		
	Odds ratio	(95%CI)	p value*	Odds ratio#	(95%CI)#	p value#*
Sex						
Male	1			1		
Female	1.27	1.21 – 1.32	<0.001	1.37	1.31 – 1.43	<0.001
Age group						
18 – 45	1			1		
45 – 70	1.18	1.12 – 1.24	<0.001	1.13	1.08 – 1.19	<0.001
70 – 99	1.35	1.28 – 1.42	<0.001	1.24	1.17 – 1.31	<0.001
Year of index prescription						
2001 -2003	1			1		
2004 – 2006	1.16	1.11 – 1.22	<0.001	1.16	1.11 – 1.21	<0.001
2007 – 2009	1.22	1.17 – 1.28	<0.001	1.23	1.17 – 1.29	<0.001
Classic Symptoms						
No	1			1		
Yes	0.95	0.89 - 1.00	0.05	0.98	0.92, 1.04	0.54
Presence of AF						
No	1			1		
Yes	1.29	1.18 – 1.41	<0.001	1.19	1.08 -1.31	<0.001
Raised blood pressure/lipids						
No	1			1		
Yes	1.30	1.25 – 1.35	<0.001	1.19	1.14 – 1.24	<0.001
Presence of Diabetes						
No	1			1		
Yes	1.62	1.51 – 1.74	<0.001	1.57	1.46 – 1.69	<0.001
Clinical reasons for TSH measurement						
Depression	1.62	1.51 – 1.74	<0.001	1.11	0.99 – 1.25	0.08
Tired	0.97	0.83 – 1.13	0.70	1.13	0.96 – 1.32	0.14
Weight gain/obesity	0.76	0.63 – 0.90	0.002	0.80	0.67 – 0.96	0.02
Peripheral Oedema	0.83	0.74 – 0.94	0.002	0.77	0.69 – 0.87	<0.001
Menstrual irregularities	0.82	0.72 – 0.93	0.001	0.89	0.79 – 1.02	0.09
Diabetes review	1.63	1.44 – 1.85	<0.001	1.20	1.05 – 1.39	0.01
General Screening	0.87	0.77 – 0.99	0.03	0.88	0.78 – 1.00	0.06

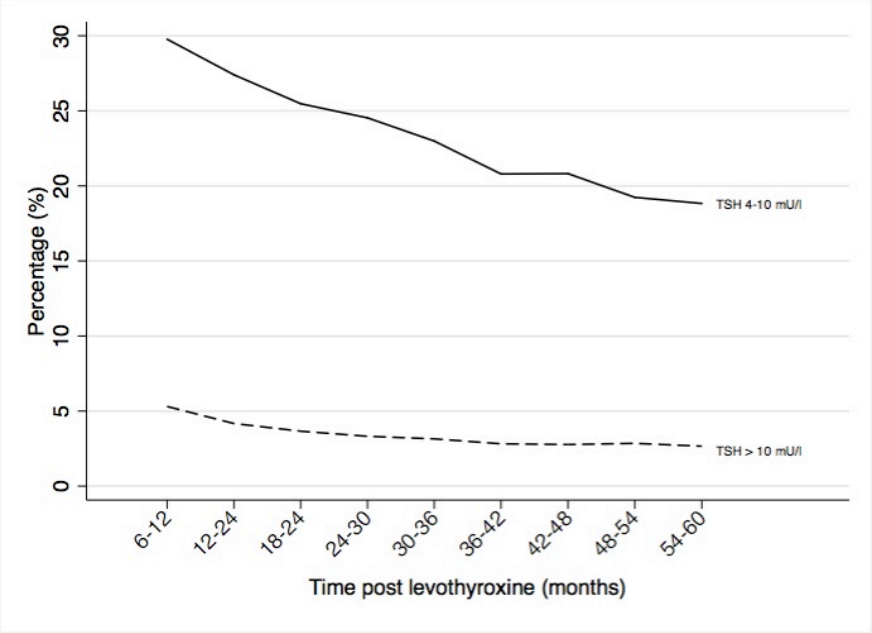
49,185 individuals in model (Individuals with TSH <4 mU/l excluded)
Adjusted for age at prescription, year of prescription presence of cardiovascular risk factors and presence of classic symptoms

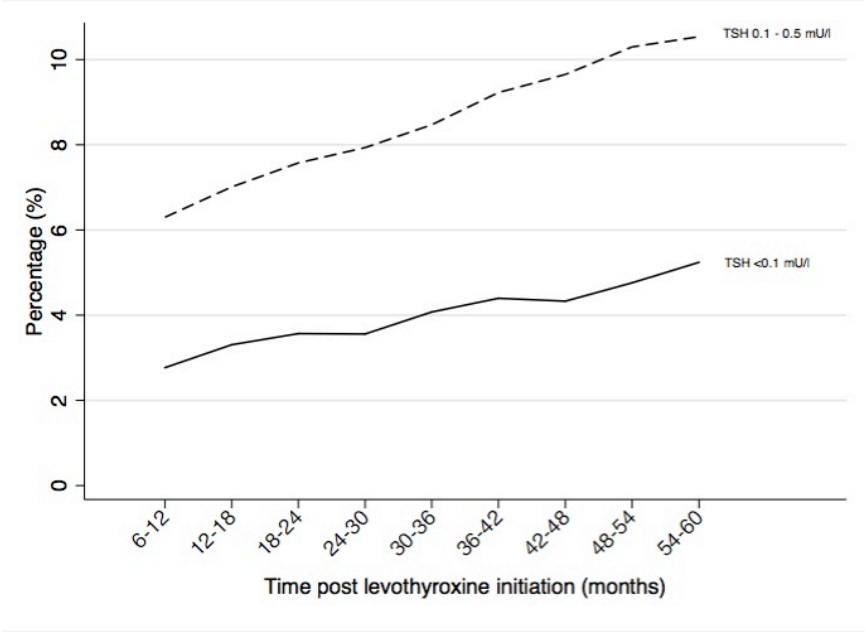
5.3.5 TSH levels post-initiation of levothyroxine

Trends are shown in TSH levels post levothyroxine (**Fig 15**). Not all individuals had TSH levels repeated regularly. The dataset was created in 2010, at which time we had TSH levels at 3 year follow-up in 17,154 individuals (51.5% of those with 3 year follow-up) and 5 year follow-up in 9,252 individuals (39.7% of those with 5 years follow-up) During the period, 6 month–5 years post levothyroxine initiation the percentage of

those with a TSH less than 0.1mU/l increased from 2.7%–5.8% and those with a TSH between 0.1-0.5mU/l increased from 6.3-10.2%; this was accompanied by a fall in those with a TSH between 5-10 mU/l from 29.8% to 18.8% (**Figure 15**). 2.7% of individuals still had a TSH greater than 10mU/l even 5 years after starting levothyroxine.

Figure 15 TSH levels after levothyroxine initiation





Individuals' baseline characteristics appeared to substantially influence the odds of developing a suppressed TSH 5 years post-levothyroxine (**Tables 37 and 38**): these included being female (OR=1.57, 95%CI 1.18, 2.08 p=0.002), presenting with tiredness (OR=1.51, 95%CI 1.13, 2.01, p=0.005), or depression (OR=1.63, 95%CI 1.02, 2.60, p=0.04) having a TSH

value less than 4mU/l (OR=1.83 95%CI 1.35, 2.47 p=<0.001) or greater than 10mU/l (OR= 2.68, 95%CI 2.07, 3.44, p=<0.001). Older age was a strong protective factor in being over-replaced and this is also a key risk factor for atrial fibrillation. Having cardiovascular risk-factors at baseline was generally associated with reduced odds of a low TSH at 5 year follow-up, although the presence of atrial fibrillation or diabetes had wide confidence intervals that included equality after adjusting for confounding variables (**Tables 37 and 38)**.

Table 37 The odds of developing a suppressed TSH 5 years post levothyroxine therapy by sex, age-group, index TSH level, presence of cardiovascular risk-factors (N=9252)

Characteristic	TSH 0.1 -0.5 mU/l						TSH < 0.1 mU/l					
	Odds ratio	(95%CI)	p value*	Odds ratio#	(95%CI)#	p value#*	Odds ratio	(95%CI)	p value*	Odds ratio#	(95%CI)#	p value#*
Sex												
Male	1			1			1			1		
Female	1.40	1.19- 1.64	<0.001	1.45	1.23 – 1.73	<0.001	1.55	1.17 – 2.04	0.002	1.57	1.18 -2.08	0.002
Age group												
18 – 45	1						1			1		
45 – 70	0.81	0.70 – 0.93	0.003	0.82	0.70 – 0.95	0.009	0.71	0.58 – 0.89	0.002	0.76	0.61 – 0.94	0.01
70 – 99	0.52	0.44 – 0.62	<0.001	0.54	0.45 – 0.65	<0.001	0.38	0.28 – 0.51	<0.001	0.41	(0.30 - 0.55)	<0.001
Year of index prescription												
2001	1			1			1			1		
2002	0.95	0.80-1.14	0.64	0.97	0.80 -1.18	0.78	1.03	0.75 – 1.39	0.87	1.06	0.78 – 1.45	0.70
2003	0.97	0.82 -1.16	0.79	0.98	0.82 – 1.18	0.86	1.30	0.98 – 1.72	0.07	1.37	1.03 – 1.82	0.03
2004	0.75	0.63 -0.90	0.002	0.78	0.65 – 0.94	0.009	0.91	0.68 – 1.22	0.53	0.97	0.72 – 1.30	0.83
TSH at index prescription												
< 4.0 mU/l	1.49	1.24 – 1.79	<0.001	1.44	1.20 – 1.72	<0.001	1.96	1.46 – 2.64	<0.001	1.83	1.35 – 2.47	<0.001
4.0 - 7.0 mU/l	1			1			1			1		
7.0 – 10.0 mU/l	1.18	0.98 – 1.42	0.08	1.19	0.99 – 1.41	0.002	1.21	0.87 – 1.69	0.24	1.22	0.88 - 1.71	0.21
10 + mU/l	2.54	2.19 – 2.94	<0.001	2.82	2.22- 2.99	<0.001	2.64	2.05 – 3.39	<0.001	2.68	2.07 – 3.44	<0.001
Presence of AF												
No	1						1			1		
Yes	0.72	0.53 – 0.98	0.04	0.87	0.63 – 1.20	0.40	0.32	0.15 – 0.68	0.003	0.42	0.20 – 0.90	0.03
Raised blood pressure/lipids												
No	1						1			1		
Yes	0.70	0.61 – 0.80	<0.001	0.81	0.71- 0.94	0.004	0.55	0.44 – 0.71	<0.001	0.68	0.53 – 0.87	0.002
Presence of Diabetes												
No	1			1			1			1		
Yes	0.63	(0.48, 0.83)	0.001	0.81	(0.61 – 1.07)	0.15	0.59	0.37, 0.95	0.03	0.78	0.48 - 1.27	0.32
T4 at levothyroxine initiation												
Normal	1			1			1			1		
Low	2.02	(1.73, 2.36)	<0.001	1.60	1.36 – 1.89	<0.001	1.81	1.41 – 2.34	0.001	1.37	1.04 - 1.81	0.02

*Calculated using the Wald test # Adjusted for sex, age group, year of index prescription, TSH at index prescription

Table 38 The odds of developing a suppressed TSH 5 years post levothyroxine therapy by potential motivation for prescription (N=9252)

Characteristic	TSH 0.1 -0.5 mU/l						TSH < 0.1 mU/l					
	Odds ratio	(95%CI)	p value*	Odds ratio#	(95%CI)#	p value#*	Odds ratio	(95%CI)	p value*	Odds ratio#	(95%CI)#	p value#*
Clinical reasons for TSH measurement												
Depression	1.91	1.41 – 2.58	<0.001	1.64	1.19 - 2.27	0.003	1.86	(1.18 - 2.95)	0.008	1.63	(1.02 - 2.60)	0.04
Tired	1.51	1.25 – 1.82	<0.001	1.56	1.28 – 1.89	<0.001	1.69	(1.27 - 2.24)	<0.001	1.51	(1.13 - 2.01)	0.005
Weight gain/obesity	1.31	1.05 – 1.63	0.02	1.26	1.00 -1.59	0.05	1.10	(0.75 - 1.62)	0.61	1.03	(0.70 - 1.51)	0.89
Peripheral Oedema	0.78	0.52 -1.17	0.23	0.86	0.57 -1.30	0.49	0.50	(0.22 - 1.14)	0.10	0.57	(0.25 - 1.29)	0.18
Menstrual irregularities	1.29	0.90 – 1.83	0.16	0.99	0.68 – 1.42	0.94	1.68	(1.01 - 2.80)	0.04	1.11	(0.66 - 1.87)	0.69
Diabetes review	0.79	0.55 -1.15	0.23	0.90	0.61 -1.32	0.58	0.66	(0.34 - 1.29)	0.23	0.79	(0.40 - 1.56)	0.50
General Screening	1.15	0.85 – 1.58	0.36	1.08	0.78 – 1.51	0.63	0.96	(0.56 - 1.66)	0.90	0.99	(0.57 - 1.72)	0.99

*Calculated using the Wald test

Adjusted for sex, age group, year of index prescription, TSH at index prescription 9,252 individuals with 5 year follow-up

5.4 DISCUSSION

Our results show that the annual rate of new levothyroxine prescriptions increased 1.74 fold over our study period. During this time there was a fall in median TSH threshold at index levothyroxine prescription from 8.67mU/l to 7.88mU/l with a 30% increase in odds of having levothyroxine initiated at a TSH level <10mU/l.

This increase in rate was not simply due to an ageing population as age-adjusted and age-stratified rates also demonstrated a rise. Furthermore, it was not due to shorter prescriptions as we only counted the first ("incident") prescription a patient ever received. An increase in case-finding due to more thyroid tests being ordered [239, 243, 247], in combination with the observed fall in TSH threshold for initiating treatment could explain this increase. Since our dataset does not contain information on individuals that never received levothyroxine, we cannot calculate the relative contribution of these two factors.

Even though it may only partly account for the overall increase in the number of people being started on levothyroxine, the reduction in TSH threshold is important as it implies the net benefits of levothyroxine therapy may be more marginal. For example, the highest age-adjusted and age- stratified rates of new levothyroxine prescribing (even with a normal FT_4) were observed in the elderly (**Table 32**) who also had the highest odds of being prescribed levothyroxine with a TSH between 4-

10mU/l (**Table 36**). This is in keeping with the highest rates of subclinical hypothyroidism being detected in the elderly [2]. However a substantial number of these prescriptions may be unwarranted as mild TSH elevations may be a normal manifestation of ageing [248]. Furthermore, there is evidence that treatment of subclinical hypothyroidism in subjects over the age of 70 has less cardiovascular benefit than in younger subjects [191]. A recent randomised clinical trial has also questioned the symptomatic benefits of treating older individuals with subclinical hypothyroidism [249].

The marked increase in new levothyroxine prescriptions since 2002 may have been an unintended consequence of the Qualities and Outcome Framework [250] which required UK primary care physicians to maintain a database of patients with hypothyroidism and monitor TSH levels annually. This may have drawn more attention to thyroid function testing and levothyroxine replacement, resulting in increased case-finding and enthusiasm to initiate therapy. New prescription rates have stabilized since 2007, despite a continued fall in median TSH, which may indicate that this enthusiasm for case-finding began to wane at this stage.

The majority of patients (61%) in our dataset were initiated on levothyroxine with a TSH level of less than 10mU/l (**Figure 13**). Furthermore in the 34,808 individuals with a FT_4 available, 31.4% had levothyroxine prescribed with a TSH level <10mU/l and a normal FT_4 despite no previous cardiovascular risk factors or classic hypothyroid

symptoms. This shows there is widespread treatment of subclinical hypothyroidism. However there is a challenge in fully interpreting this data as data on thyroid antibody status were not available and we could not identify symptoms relevant to hypothyroidism prior to the TSH test resulting in the index levothyroxine prescription in 47.9% of individuals.

A summary of the guidelines for treating subclinical hypothyroidism is shown below in **Table 39**.

Table 39 International guidelines for treating subclinical hypothyroidism

Guideline	Subclinical Hypothyroidism Recommendation	Evidence Grade B
AACE[85]	Treatment based on individual factors for patients with TSH levels between the upper limit of a given laboratory's reference range and 10 mIU/L should be considered particularly if patients have symptoms suggestive of hypothyroidism, positive TPOAb or evidence of atherosclerotic cardiovascular disease, heart failure This guideline also highlighted the need for prospective intervention studies	Intermediate
ETA[251]	In younger patients (<65 years) with symptoms suggestive of hypothyroidism, a trial of levothyroxine replacement therapy should be considered Age-specific reference ranges for serum TSH should be considered in order to establish a diagnosis of subclinical hypothyroidism in older people. The oldest old subjects (>80–85 years) with elevated serum TSH ≤10 mU/l should be carefully followed with a wait-and-see strategy, generally avoiding hormonal treatment.	Intermediate

From our data FT_4 values were available in 68.3% individuals prescribed levothyroxine with a TSH between 4-10mU/l and 82.7% of this group had FT_4 values within the reference range, consistent with a diagnosis of subclinical hypothyroidism (**Table 35**). The evidence for clinical benefit of treatment in this range outside of pregnancy is weak [85].

Only 39.4% of individuals prescribed levothyroxine for subclinical hypothyroidism had a history of hypertension, raised lipids, atrial fibrillation or diabetes before levothyroxine initiation with 46.9 % having either these cardiovascular risk-factors or documented symptoms consistent with hypothyroidism prior to levothyroxine. Although some data may be unrecorded, it suggests that up to 50% of individuals with subclinical hypothyroidism are treated outside of guidelines. However it is somewhat reassuring that individuals with cardiovascular risk-factors were preferentially initiated on levothyroxine in the TSH 4-10mU/l group compared to those without cardiovascular comorbidities. Furthermore individuals over the age of 70 had high rates of being prescribed levothyroxine, even if they had a normal FT4 levels indicating there was no clear avoidance of prescribing for subclinical hypothyroidism in this age group contrary to ETA guidance[251]. However it should be noted that this guideline came out after our dataset ended. Recently a trial of levothyroxine therapy for subclinical hypothyroidism in older individuals (age > 65 years) did not find any evidence of benefit in terms of hypothyroid symptoms, mood or cognition [249]

Another concern is that contrary to ATA guidelines[81, 85] 34.6% of individuals prescribed levothyroxine with a TSH level between 4-10mU/l only had one abnormal TSH reading before initiating therapy. Greater use of confirmatory testing might reduce unnecessary prescriptions given that 46% of individuals with a TSH between 4.5-7.0mU/l reverted to normal within 2 years without treatment[252]. This is especially relevant

since the indication for levothyroxine is rarely reviewed once started. In our dataset over 90% of individuals were still being prescribed levothyroxine at the end of the study. It is quite likely that a substantial proportion of individuals on levothyroxine, would have normal thyroid function if levothyroxine was stopped. The fact that levothyroxine is easy to prescribe and inexpensive has encouraged a low threshold for its use.

Set against the uncertain potential for benefit in a large proportion of patients initiated on levothyroxine, it is important to examine the potential for harm. 5 years after levothyroxine initiation 10.2% of patients had a low TSH and 5.8% had a suppressed TSH. Individuals with a suppressed TSH are at a potentially increased risk of developing osteoporotic fractures [246] and atrial fibrillation [253] and data for the increased risk of harm from subclinical hyperthyroidism are stronger than the data of potential benefit from treatment of subclinical hypothyroidism. However it should be highlighted that individuals on excess levothyroxine treatment will have relatively lower FT3 levels than people with subclinical hyperthyroidism, so it is difficult to draw a direct comparison between subclinical hyperthyroidism and excess levothyroxine therapy.

Individuals with cardiac risk factors had reduced odds of developing a suppressed TSH, suggesting that prescribers were aware of this risk, but 10.6% of individuals treated for subclinical hypothyroidism who had cardiovascular risk factors ended up with a low TSH level which may have

actually increased their risk. Individuals with tiredness or depression at baseline but not those with diabetes or obesity were more likely to be over-replaced at 5 years (**Table 38**), raising the possibility that there may be an element of intentional increased dosing with levothyroxine rather than a lack of careful monitoring in these individuals.

There are now 1.6 million individuals in the UK on long-term levothyroxine most of whom have been prescribed it for primary hypothyroidism [238]. If current practice continues, up to 30% of people on levothyroxine may have been prescribed it without an accepted indication, and with potential for net harm if they develop even a low TSH (as occurred in12.2% of individuals prescribed levothyroxine for subclinical hypothyroidism in our dataset). Effects may be substantial in the western world. In the USA the prevalence of hypothyroidism is similar to the UK [42] and one might therefore expect approximately 5 million individuals in the USA to be on long-term levothyroxine for primary hypothyroidism; if prescribing patterns in the USA are similar over 1.6 million individuals may be on levothyroxine with limited evidence of benefit.

The strengths of our study include the use of a large population-based dataset from many different practitioners collected over a long period. Detailed clinical data allowed us to ascertain cases of primary hypothyroidism and exclude individuals who had levothyroxine prescribed as a result of pregnancy or following treatment of hyperthyroidism or

pituitary disease. In addition, the use of electronic records by UK primary care physicians to issue prescriptions makes it unlikely that prescriptions of levothyroxine were missed. Similarly, almost all laboratories sent biochemical data electronically by 2000, so few TSH results were unavailable and transcription errors were eliminated. We also had substantial data on cardiovascular risk-factors and symptoms prior to levothyroxine initiation to enable us to investigate the appropriateness of levothyroxine prescriptions.

Patients prescribed levothyroxine with a normal TSH level accounted for 6% of prescriptions. As patients needed at least 12 months of up to CRPD standard follow-up before levothyroxine initiation to be included it cannot be explained by patients with established hypothyroidism on levothyroxine moving to a CPRD practice. A proportion of individuals might be planning pregnancy, but never having a pregnancy thereby being missed by our pregnancy CPRD codes. The majority of this prescribing levothyroxine with a normal TSH is likely to reflect poor practice, but from our data it is unclear if this was a problem in only certain parts of the UK. Nevertheless, retrospective studies of individuals who have been started on levothyroxine at a normal TSH would be important to clarify risk benefits of this practice and also see if there were underlying reasons (e.g. genetic or environmental) for hypothyroid symptoms at normal TSH levels.

Although the use of CPRD has provided substantial data there are key limitations of this resource. As data is routinely collected, rather than as part of a protocol missing data is an inevitable consequence. This is highlighted by the large number of individuals which did not have regular TSH levels measured following levothyroxine imitation. Furthermore absence of a read code for a disease is interpreted as absence of the disease itself therefore whilst the positive predictive value of a read code is high sensitivity is lower. Data from secondary care must be manually entered. Given hyperthyroidism is usually managed in secondary care, this may lead to people with treated hyperthyroidism, being labelled as primary hypothyroidism. Data on levothyroxine adherence is also not available. The key limitations in this study were the lack of data on individuals who did not receive a levothyroxine prescription and the lack of reliable data on thyroid peroxidase antibody titres. Furthermore data on FT_4 measurements were not available in all subjects, as this estimation is not always routine practice and follow-up TSH values were only available in 40% of the cohort at 5 years. Hence there is the potential for bias in the subsection of subjects analysed however there was no observed difference in sex or age-group between those with FT_4 levels available and those without. The TSH assay used varied between laboratories, and we were unable to account for this, although the majority of assays have similar thresholds for defining low or suppressed TSH. Finally, we were unable to identify and exclude from our denominator data individuals who were prescribed levothyroxine prior to 2001 (and hence not at risk of receiving another first thyroxine

prescription) We were also not able to remove from the denominator person-years for individuals excluded by GPRD in the creation of our dataset. However we consider that the impact of this on the accuracy of our results is likely to be small, particularly with regard to the relative rate.

In summary, my work suggests there is widespread prescribing of levothyroxine for borderline TSH levels where there is limited evidence of benefit. This practice may even be harmful, given the relatively high risk of developing a suppressed TSH after treatment. A key future study here would be to assess whether the potential benefits of treating borderline hypothyroidism in younger adults (age < 65 years) can be realized without over treatment. Whilst thyroidologists are still debating whether subclinical hypothyroidism should be more widely treated, it is increasingly apparent that this is already happening in primary care. Randomised controlled trials with sufficient power to assess the health consequences of borderline/subclinical hypothyroidism and its treatment are urgently needed to refine current levothyroxine prescribing and indicate the balance of risks and benefits of current practice. There remains attractive potential benefit in the potential for cardiovascular outcome improvement in younger individuals, although neuro-psychological benefits of treating borderline low thyroid function are much less clear. Many young women are started on levothyroxine and they may have less cardiovascular benefit as males as they are of lower risk, but may be more susceptible to adverse bone outcomes. However

the widespread use of levothyroxine in women of child-bearing age may have implications for the optimal management in pregnancy, given clear potential benefits of optimal thyroid hormone replacement during pregnancy, this raises key questions regarding whether universal thyroid screening in pregnancy is warranted. Both of these important issues will be covered in the next 2 chapters.

Chapter 6 TSH levels and risk of miscarriage in women on long-term levothyroxine data from CPRD

In the previous chapter I identified changing TSH thresholds for levothyroxine initiation for primary hypothyroidism. This has resulted in substantial numbers of women of child-bearing age being established on levothyroxine. Given the importance of thyroid status on pregnancy outcomes the management of women on levothyroxine during their pregnancy is of paramount importance. Given the UK has a high burden of hypothyroidism and borderline iodine sufficiency, screening and treating for low thyroid function may result in substantial obstetric benefits. It is therefore informative to study outcomes by TSH level in women already established on levothyroxine. In this chapter I explore the adequacy of current management of women established on levothyroxine prior to pregnancy and how this might influence obstetric outcomes. Here I used primary care data to provide a substantial number of women established on levothyroxine who became pregnant as studies based on single hospital clinics have had insufficient power.

6.1 INTRODUCTION

Primary hypothyroidism affects 3-10% of women [34, 42], and is predominantly managed in primary care [238]. A substantial proportion of affected individuals are of childbearing age [34, 42] and approximately 1-2% of women receive levothyroxine during pregnancy [4, 94]. There is an estimated 30-50% increase in levothyroxine requirements during

pregnancy [254, 255] and most hypothyroid women who become pregnant will require an increase in levothyroxine dose although the optimal magnitude and timing of this increase remains uncertain [94, 255, 256].

Recent reports have highlighted that between 24–55% of women established on levothyroxine prior to pregnancy have an elevated thyroid stimulating hormone (TSH) at their first antenatal visit [257-262]. Suboptimal thyroid function is associated with adverse pregnancy outcomes including an increased risk of miscarriage, premature birth, gestational hypertension, placental abruption, postpartum hemorrhage [113, 263, 264] as well as impaired neurological development in the offspring [105, 265]. As would be expected these complications are more common and severe in overt hypothyroidism than in subclinical hypothyroidism [95, 266]. A recent systematic review reported that levothyroxine is effective at lowering the risk of preterm delivery (RR: 0.41, 95%CI: 0.24, 0.68) and miscarriage (RR: 0.19, 95%CI: 0.08, 0.39) in overt hypothyroidism [267].

In 2007, the Endocrine Society recommended that hypothyroid women contemplating pregnancy should have their levothyroxine dose adjusted to achieve a preconception TSH <2.5mU/L [268]. Additional monitoring and dose titrations are also advised to maintain a TSH between 0.2–2.5 mU/l in the first trimester and between 0.3–3.0 mU/l in later pregnancy [268]. Similar targets have been endorsed by subsequent guidelines [93, 269]. However inadequate treatment of primary hypothyroidism remains

a major problem with 40-48% of hypothyroid patients either over-treated or under-treated [42, 189, 270, 271] from large regional case series.

To date there has been no population-based study of pregnancy outcomes in women on long-term levothyroxine. Women with thyroid function available in population birth cohorts are likely to be different to women established on levothyroxine, even if TSH levels are similar, as women on levothyroxine, will likely have lower FT3 levels and be much more likely to be TPO antibody positive. TPO positivity in particular is associated with an impaired thyroidal response to hCG [272].

The majority of studies in this area have been small and in groups of women attending specialist hospital antenatal clinics [257-259, 262]. These hospital cohorts are less representative of the general population and might underestimate the incidence of early miscarriages occurring prior to specialist clinic enrollment. In this analysis, I addressed this topic using data from the CPRD as described in the previous chapter. My aim was to determine the adequacy of thyroid hormone replacement in pregnancy and to examine pregnancy outcomes in relation to TSH levels.

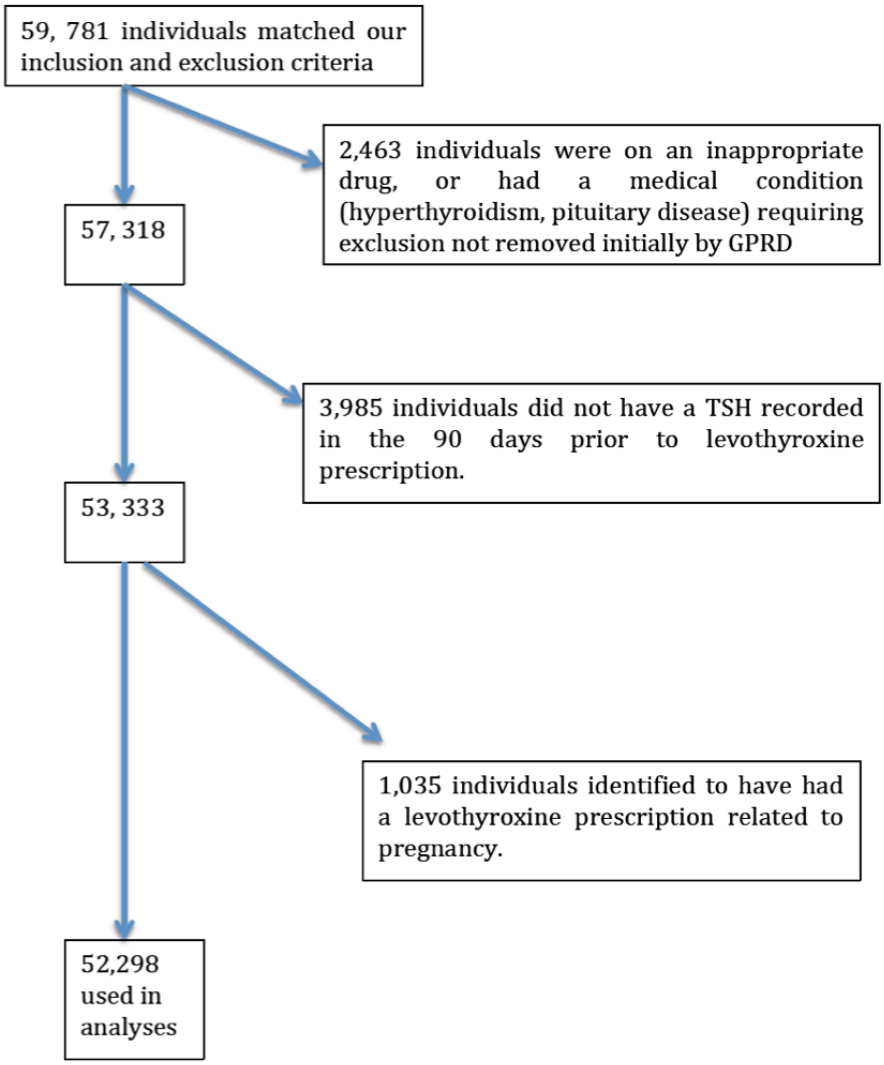
6.2 METHODS

6.2.1 Pregnancy Cohort

The cohort was based on our CPRD dataset previously described in **Chapter 2** and **Chapter 5** summarized in **Figure 16**. This contained detailed primary care clinical data including patient symptoms, outcomes

and biochemical measures on over 50,000 patients on levothyroxine. In this analysis we identified 7,978 women of childbearing age (18-45 years) and from these we identified 1,035 pregnancies of which 1,013 were completed pregnancies (foetal loss or birth before the end of the dataset records) in women established on levothyroxine for at least 6 months.

Figure 16 CPRD dataset of primary hypothyroidism



6.2.2 Identification of pregnancies and TSH levels

Pregnancies were identified using 987 pregnancy related codes including pregnancy confirmation tests, antenatal clinic appointments, and

delivery records provided by Caroline Minassian (London School of Hygiene and Tropical Medicine). Codes were used in combination to estimate the date of pregnancy and thereby the timing of TSH tests. For instance, codes pertaining to confirmation of pregnancy, early stage of pregnancy and morning sickness were used to identify first trimester dates whilst codes relating to delivery dates and antenatal clinic attendance from 12-40 weeks were used to confirm second and third trimester dates. Pregnancy dates were linked to date of first levothyroxine prescription and only a woman's first pregnancy occurring at least six months after levothyroxine initiation was included. If individuals had more than one TSH level recorded in a trimester the highest reading was used. Individuals with evidence of a pregnancy but no evidence of an outcome such as delivery, miscarriage or termination were excluded from pregnancy outcome analysis (delivery/miscarriage), but not from descriptive analyses of TSH levels during pregnancy. Sensitivity analyses were undertaken with individuals without a delivery outcome (miscarriage/termination aside) recoded as a successful delivery. There was insufficient data on free thyroxine levels and thyroid peroxidase antibody titres so these were not analysed.

6.2.3 Identification of adverse pregnancy outcomes

Adverse pregnancy outcomes were identified using medical codes (**Appendix 2**). For the primary analysis codes relating to miscarriages and stillbirths were used. For a secondary analysis, other adverse pregnancy complications were identified and grouped together using medical codes,

specified *a priori* covering emergency caesarean section, pre-eclampsia, post-partum hemorrhage, placental abruption, prematurity, low birth weight, growth restriction, need for intensive care, and neonatal death.

6.2.4 Identification of diabetes and socio-economic status

Individuals with a diagnosis of type-1diabetes, type-2 diabetes or gestational diabetes were identified using multiple medical codes pertaining to these conditions and were only included if diabetes preceded or occurred during the pregnancy of interest. Quintiles of socio-economic status were calculated from the Index of Multiple Deprivation for the postcode of each individual's general practice.

6.2.5 Statistical analysis

Serum TSH is presented as median (inter-quartile range). TSH was compared according to the year of pregnancy before and after the Endocrine Society guidelines [268] (2001-2007 versus 2008-2009) and by pregnancy trimester (1st versus 2nd/3rd) using the Wilcoxon-rank test. The primary analysis assessed the odds of miscarriage/stillbirth by first trimester TSH level. Secondary analyses were undertaken to examine the odds of other pregnancy complications by first and second/third trimester TSH. To reflect trimester specific reference-ranges as recommended by current international guidelines [93, 269] first trimester TSH levels were split into 5 categories: i) <0.2 mU/l) ii) 0.2–2.50 mU/l iii) 2.51–4.50 mU/l iv) 4.51–10 mU/l v) >10 mU/l. The lower three TSH level categories were subtly different for second/third trimester analysis, i) <0.3 mU/l, ii) 0.3-3.00 mU/l iii) 3.01-4.50 mU/l (26, 27. At the time of

this analysis the 0.2-2.5 mU/l and 0.3-3.0 mU/l categories represented the recommended optimal ranges for the first and second/third trimesters respectively and were used as the reference category for the multivariable model. Analyses were adjusted for age, year of pregnancy, social class and diabetes before or during pregnancy

6.3 RESULTS

6.3.1 TSH levels in women of child-bearing age (18-45 years) on levothyroxine (N=7,978).

The CPRD dataset of 52,298 patients on levothyroxine for primary hypothyroidism was interrogated. 7,978 women aged 18-45 years who had been on levothyroxine for at least one year had a TSH level available. Analysis of this TSH measurement, revealed a median TSH of 2.22 mU/l (IQR 0.97–3.78) with 3,678 (46.1%) having a TSH > 2.5 mU/l and 364 (4.6%) with TSH > 10 mU/l. 1,082 women (13.6%) were over-treated with a TSH <0.4 mU/l and 408 women (5.11%) had a TSH < 0.1 mU/l. Data are summarized in **Table 40**.

Table 40 Latest TSH levels in women established on levothyroxine for at least one year (N=7,978)

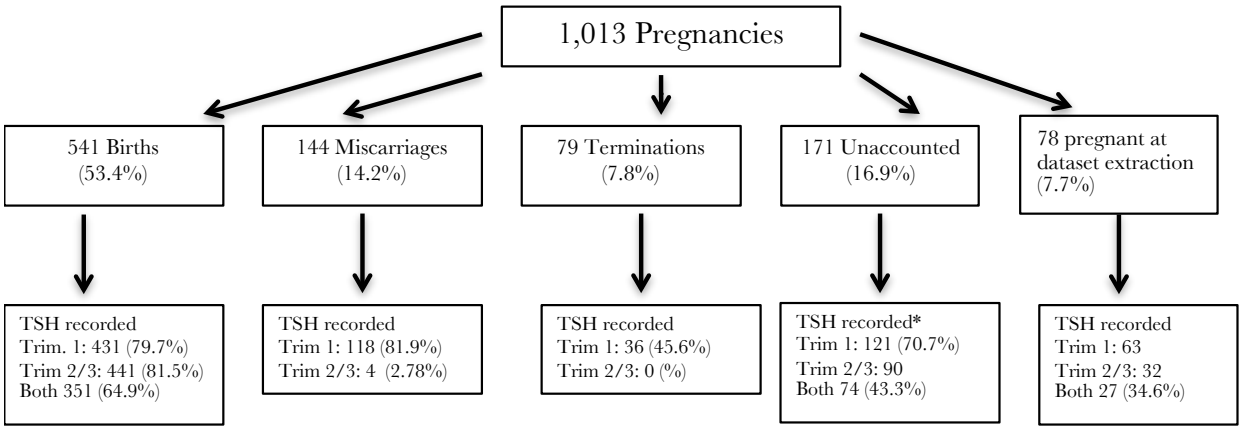
TSH threshold (mU/l)	Number of individuals	(%)
<0.02	167	2.1
0.02 – 0.40	915	11.5
0.40 – 2.50	3,361	42.1
2.50 – 4.50	2,127	26.7
4.50 -10.0	1,044	13.1
>10.0	364	4.56

1,082 women (13.6%) of women had a TSH < 0.4 mU/l
408 women (5.11%) of women had a TSH < 0.1 mU/l

6.3.2 TSH levels in women who became pregnant (N=1,013)

The median age at conception was 33 years (IQR 29-37) with a median duration of levothyroxine therapy prior to pregnancy of 17.5 months (IQR 11.2–25.8). Of the 1,013 pregnancies, we identified 541 deliveries (53.4%), 144 miscarriages (2 were stillbirths) (14.2%), 79 terminations of pregnancy (7.8%), 171 pregnancies with no outcome recorded (16.9%) and 78 pregnancies (7.7%) which were ongoing when the data were extracted (before the completion of pregnancy) (**Figure 16**). No differences were observed between individuals with pregnancy outcomes unaccounted for and those with pregnancy outcomes accounted for with regard to calendar year of pregnancy (p=0.18), age at pregnancy (p=0.17), first trimester TSH level (p=0.17) or second/third trimester TSH level (p=0.73) presence of diabetes (p=0.37) or social class (p=0.23).

Figure 17 Pregnancy outcomes and TSH measurements in CPRD pregnancy dataset



Trim= Trimester

880 women (86.9%) had a TSH level recorded during pregnancy while 769 (75.9%) had a TSH recorded in the first trimester. Of women with a first trimester TSH, 434 (56.4%) had levels that were greater than 2.5 mU/l, 224 (29.1%) were greater than 4.50 mU/l and 57 (7.41%) were greater than 10 mU/l. The spread of first trimester TSH values by category is shown in **Figure 18**.

Figure 18 Highest recorded TSH levels during trimester 1

Numbers of women in each category are shown above each bar. Percentages are derived from all women with a documented TSH in that category

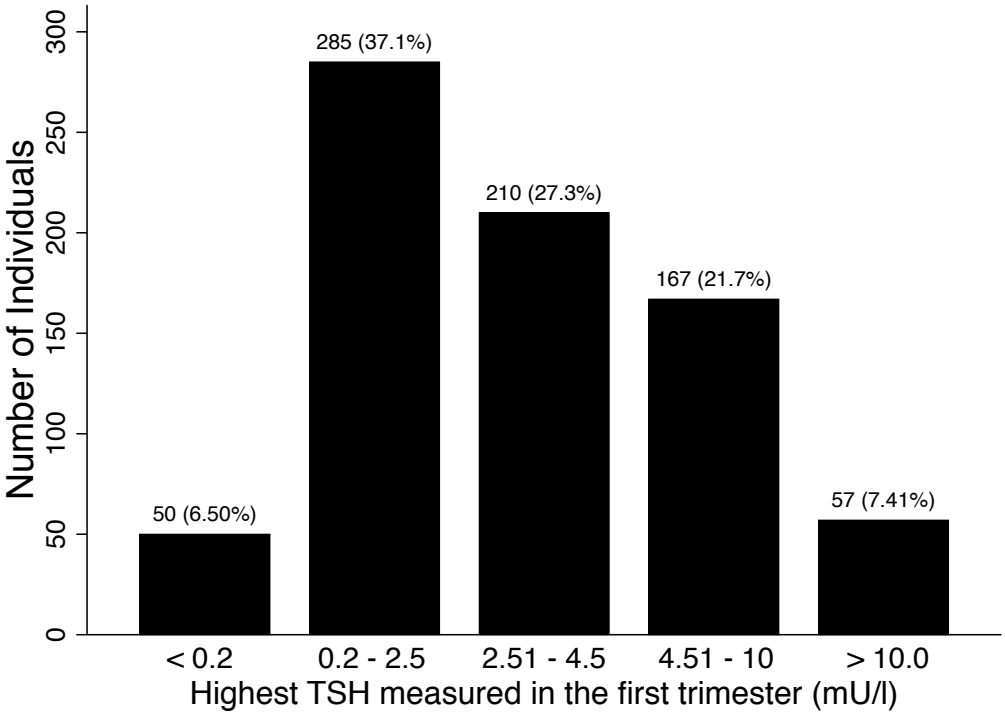
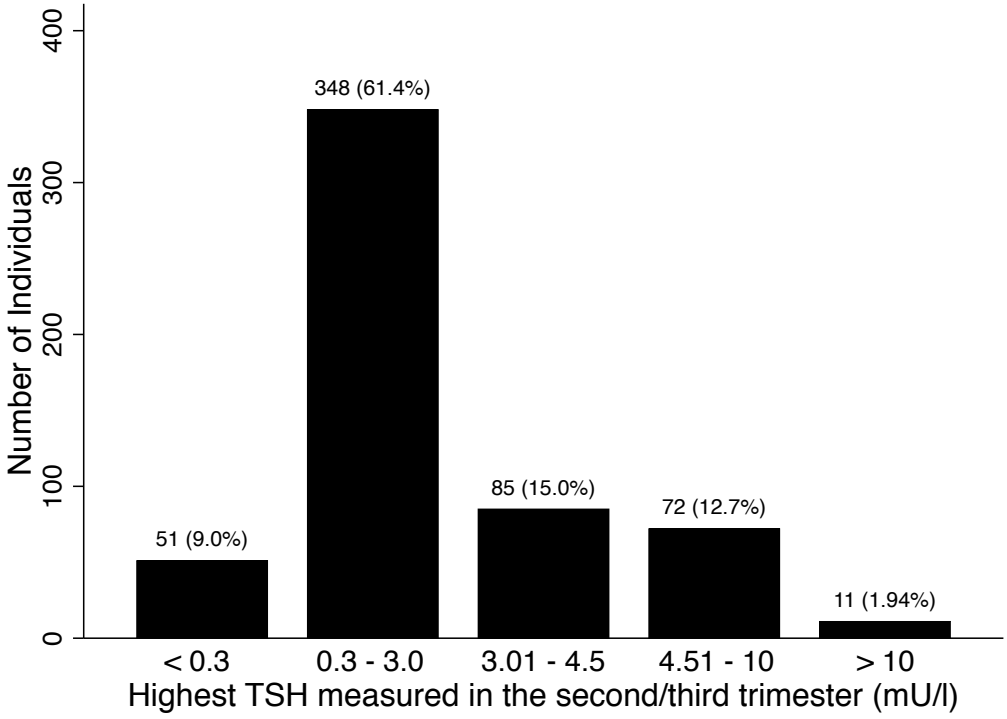


Figure 19 Highest recorded TSH levels during trimesters 2 and 3

Numbers of women in each category are shown above each bar. Percentages are derived from all women with a documented TSH in that category



Median TSH in the first trimester was slightly lower before 2007 (2.78 mU/, IQR 1.33-4.96) than after 2007 (2.98 mU/l, IQR 1.77-5.31) p=0.09.

A summary of TSH levels by year-group is shown in **Table 41**.

Table 41 Gestational TSH levels by year of pregnancy in women on levothyroxine

Trimester	Year group	Number of women	Median TSH mU/l	IQR
1	2001-2003	98	2.10	0.86 - 4.33
	2004-2006	294	2.97	1.48 – 5.01
	2007-2009	377	2.98	1.77 – 5.31
2/3	2001-2003	80	2.34	1.79 - 3.68
	2004-2006	218	2.01	0.99 - 2.05
	2007-2009	269	2.09	1.20 – 3.20

769 individuals with TSH measured during first trimester
567 individuals with TSH measured during second/third trimester

567 women had TSH measured in the 2nd/3rd trimester of which 348 (61.4%) had a TSH within the trimester-specific target range of 0.30-3.0 mU/l. In addition, 168 women (29.6%) had TSH >3.0mU/l in the 2nd/3rd trimester and 51 (9.0%) had a TSH <0.3 mU/l (**Figure 19**). Median TSH levels were lower in the 2nd/3rd trimester (2.10 mU/l, IQR 1.19–3.37 mU/l) than in the first trimester (2.89 mU/l, IQR 1.50–5.0 mU/l) (p=<0.0001). However inadequate TSH levels persisted in a substantial proportion of pregnancies and 66.5% of women with TSH >2.5 mU/l in the first trimester who also had a TSH measured in the 2nd/ 3rd trimesters, had a 2nd/3rd trimester TSH greater than the target of 3.0mU/l. 133 (13.1%) of the 1,013 pregnancies had no corresponding TSH record and over half of these (51.9%) ended in miscarriage or termination. A small number of women amounting to 20 (3.7%) out of the 541 women with a delivery at term recorded had no corresponding TSH measurement over

the entire duration of pregnancy and thus did not appear to have had thyroid function measured during pregnancy despite being established on levothyroxine.

6.3.3 Delivery/Miscarriage outcomes by first trimester TSH

We identified 431 deliveries and 118 miscarriages in the 769 pregnancies of women with a TSH level recorded in the first trimester. In 22 of the 144 total miscarriages (15.3%) the miscarriage was the first GPRD record of a pregnancy and was not preceded by a thyroid function test in pregnancy.

Median 1st trimester TSH was higher in women who miscarried than in those with a successful delivery 3.59mU/l vs. 2.80mU/l (p=0.003). After adjusting for maternal age, calendar year, social class and presence of diabetes, the odds of miscarriage rose with increasing TSH levels above the target TSH range of 0.2-2.5 mU/l (p for trend =0.008) with the greatest impact observed with TSH levels greater than 10 mU/l OR=3.95 (95%CI 1.87, 8.37) (**Table 42**). An increase in the odds of miscarriage was also observed with TSH levels between 4.51–10 mU/l (OR=1.80, 95%CI 1.03, 3.14). In individuals with TSH 0.2-2.5 mU/l the risk of miscarriage was 17%, rising to 30% at TSH >4.5 mU/l and 41.5% at TSH >10mU/l. Individuals with a maximum TSH <0.2 mU/l or TSH 2.51–4.5 mU/l had an odds ratio of miscarriage greater than 1 but this is unlikely to be of any clinical relevance.

In addition, 60 women had a recorded TSH <0.2 mU/l which did not persist through pregnancy. Analysis of these individuals with transient TSH suppression did not reveal any clear increase in the odds of miscarriage compared to individuals who never had had a TSH outside the trimester-specific target range OR=0.62 (95%CI 0.25, 1.54) p=0.30. Sensitivity analyses with all unidentified pregnancy outcomes recoded as a successful delivery revealed similar associations (**Table 42**).

Table 42 Odds of miscarriage by 1st trimester serum TSH level

TSH (mU/l)	N	Foetal loss N	Foetal loss (%)	Odds Ratio	95%CI	p	OR	95%CI	P
<0.2	36	6	16.7	0.97	0.37-2.51		1.14	0.62-1.93	
0.2-2.5	199	34	17.1	1			1		
2.51-4.5	151	29	19.2	1.15	0.66-2.00	0.02	1.09	0.61-1.93	0.008
4.51-10	122	32	26.2	1.73	1.00, 2.98		1.80	1.03-3.14	
>10	41	17	41.5	3.44	1.66, 7.08		3.95	1.87-8.37	

549 individuals in model: 431 deliveries 118 miscarriages
*Adjusted for age, calendar year of pregnancy, diabetes during or before pregnancy, social class
Reference category is the recommended 1st trimester TSH: 0.2-2.5 mU/L
Test for trend comparing the odds of miscarriage by TSH levels above 2.5 mU/l to the reference category of 0.2-2.5 mU/l.

Table 43 Odds of miscarriage by 1st trimester serum TSH assuming all unidentified deliveries resulted in a successful outcome

TSH (mU/l)	Total (N)	Miscarriages (N)	Odds of Miscarriage	95%CI	p#	Odds of Miscarriage*	95%CI*	p#*
<0.2	48	6	1	0.40, 2.52		0.98	0.38, 2.55	
0.2-2.5	272	34	1		0.001	1		<0.001
2.51-4.5	200	29	1.19	0.70, 2.02		1.09	0.63, 1.89	
4.51-10	160	32	1.75	1.03, 2.97		1.73	1.02, 2.96	
>10	53	17	3.31	1.68, 7.08		3.64	1.81, 7.32	

733 individuals in model: 615 deliveries 118 miscarriages *Adjusted for age, calendar year of pregnancy, diabetes during or before pregnancy, social class Reference category is the recommended 1st trimester TSH: 0.2-2.5 mU/L
Test for trend comparing the odds of miscarriage by TSH levels above 2.5 mU/l to the reference category of 0.2-2.5 mU/l.

Analyses stratified by age revealed that in women aged <35 years sub-optimal thyroid function was associated with higher risk estimates of miscarriage than in women aged 35 years or older. This may also reflect the greater likelihood of women being TPO antibody positive being diagnosed at an earlier age. Alternatively, this may be related to the higher baseline risk of miscarriage in the older population (**Table 44**).

Table 44 Analysis of the odds of miscarriage by first trimester TSH levels stratified by age

	Age <35 years					Age 35 years and older				
TSH (mU/l)	Total (N)	Miscarriages (N)	Odds of Miscarriage	95%CI	p#	Total (N)	Miscarriages (N)	Odds of Miscarriage*	95%CI*	p#*
<0.2	20	4	2.32	0.65, 8.33		16	2	0.54	0.11, 2.73	
0.2-2.5	116	12	1		<0.001	83	22	1		0.29
2.51-5.0	90	11	1.16	0.48, 2.81		61	18	1.06	0.49, 2.29	
5.01-10	67	13	2.19	0.92, 5.21		55	19	1.50	0.71, 3.19	
>10	23	11	8.84	3.09, 25.2		18	6	1.67	0.52, 5.27	

*Adjusted for age, calendar year of pregnancy, diabetes during or before pregnancy, social class
Reference category is the recommended 1st trimester TSH: 0.2-2.5 mU/L
Test for trend comparing the odds of other adverse events† by TSH levels above 2.5 mU/l to the reference category of 0.2-2.5 mU/l.

6.3.4 Odds of other adverse pregnancy outcomes by 1st trimester and 2nd/3rd trimester TSH

Out of the 431 births with a TSH level measured in the first trimester, 29 (6.73%) had other adverse pregnancy outcomes. Of the 441 births with a TSH level measured in the second/third trimester 31 (7.0%) had other adverse pregnancy outcomes. There was no clear pattern of association with adverse events around delivery and TSH level in the first trimester or second/third trimester (**Table 45 and Table 46**) although individuals with a TSH level in the target ranges had the lowest odds of a late adverse pregnancy outcome.

Table 45 Odds of other adverse obstetric outcomes by 1st trimester serum TSH level

TSH (mU/l)	Total (N)	Adverse Outcomes (N)	Unadjusted odds of late adverse outcomes	95%CI	p#	Adjusted odds of late adverse outcomes*	95%CI*	p#*
<0.2	26	4	3.47	0.95, 12.7		3.44	0.89, 13.3	
0.2-2.5	165	7	1		0.21	1		0.19
2.51-4.5	122	8	1.58	0.56, 4.49		1.56	0.54, 4.47	
4.51-10	90	9	2.51	0.90, 6.98		2.58	0.92, 7.26	
>10	23	1	0.98	0.12, 8.34		1.05	0.12, 8.96	

426 individuals in model: 29 with adverse outcomes

*Adjusted for age, calendar year of pregnancy, diabetes during or before pregnancy, social class
Reference category is the recommended 1st trimester TSH: 0.2-2.5 mU/L
Test for trend comparing the odds of other adverse events† by TSH levels above 2.5 mU/l to the reference category of 0.2-2.5 mU/l.
† emergency caesarean section, pre-eclampsia, post-partum hemorrhage, placental abruption, prematurity, low birth weight, growth restriction, need for intensive care, and neonatal death.

Table 46 Odds of other adverse obstetric outcomes† by 2nd/3rd trimester serum TSH level

TSH (mU/l)	Total (N)	Adverse Outcomes (N)	Unadjusted odds of Adverse Outcomes	95%CI	p#	Adjusted odds of Adverse Outcomes*	95%CI*	p#*
<0.3	37	4	2.08	0.65, 6.66		2.64	0.80, 8.67	
0.3-3.0	273	15	1		0.20	1		0.11
3.01-4.5	64	6	1.77	0.56, 4.57		1.85	0.68, 5.08	
4.51-10	59	5	1.59	0.28, 21.3		1.88	0.64, 5.54	
>10	7	1	2.45	0.65, 6.65		3.36	0.37, 30.3	

441 individuals in model: 31 with adverse outcomes
*Adjusted for age, calendar year of pregnancy, diabetes during or before pregnancy, social class
Reference category is the recommended 1st trimester TSH: 0.3-3.0 mU/L
Test for trend comparing the odds of other adverse events† by TSH levels above 3.0 mU/l to the reference category of 0.3-3.0 mU/l.
† emergency caesarean section, pre-eclampsia, post-partum hemorrhage, placental abruption, prematurity, low birth weight, growth restriction, need for intensive care, and neonatal death.

7.4 DISCUSSION

This analysis of a primary care dataset studied TSH levels and birth outcomes during pregnancy in women established on levothyroxine for primary hypothyroidism. It showed that almost half of women of reproductive age who take levothyroxine for primary hypothyroidism have a thyroid status that is not optimal for pregnancy according to current guidelines. Furthermore, up to 60% of pregnant women have suboptimal TSH levels in early pregnancy. In addition, I found no evidence of improvement in gestational thyroid hormone replacement since the Endocrine Society guidelines were introduced in 2007. Our findings are in keeping with recent regional data from Scotland [260] and Wales [262] and suggest that the current problem is widespread and persistent. Recent ATA guidelines [93] encourage women having the confidence to independently increase their levothyroxine dose on confirmation of pregnancy. However for the UK greater awareness of this for general practitioners is essential as they will provide the medical input in early pregnancy - a time where thyroid status is particularly important.

This is an important issue to address as I also observed that TSH levels above 2.5 mU/l in the first trimester were associated with increased odds of miscarriage with levels between 4.51-10 mU/l having almost double and levels >10mU/l nearly a fourfold increase in the odds of subsequent miscarriage even after adjusting for key confounders. In effect women with TSH levels within the current guideline targets (0.2-2.5 mU/l) had the lowest miscarriage rates (17]. It would therefore seem reasonable

that the currently recommended preconception and early gestation TSH targets (<2.5 mU/l) are maintained [4].

Although I observed a trend towards increasing odds of miscarriage with rising TSH above 2.5 mU/l I did not find a clear increase in the odds of miscarriage in women with TSH levels 2.51-4.5 mU/l. This finding is in contrast to the large (N=4,123) population based study by Negro *et al* which reported an increased risk of miscarriages at TSH levels 2.5-5.0 mU/l [148] although these women were not on levothyroxine. In addition, the miscarriage risk in women with a TSH of 2.5-4.5 mU/l was higher than that observed by Negro *et al* in women with a comparable TSH of 2.5-5.0 mU/L (19% vs. 6%). The reasons for these differences are unclear but the higher miscarriage rates in my analysis may have arisen because the Negro study was restricted to antibody negative women while our patients were older (median age 33 years vs. 28 years) and more likely to be antibody positive from Hashimoto's thyroiditis, all factors that are known to increase the risk of pregnancy loss [273]. Furthermore this study may have been underpowered to detect an effect within this TSH category.

My data also indicate the need for a more meticulous approach to thyroid hormone replacement in pregnancy. Around 13% of pregnant women in this cohort did not appear to have a TSH level measured throughout pregnancy highlighting the need for closer monitoring. Another important observation was that transient TSH suppression did not carry an increased

risk of miscarriage suggesting that brief periods of over-replacement were not detrimental to obstetric outcomes and should not deter judicious increases in levothyroxine dose to attain target TSH levels. However larger observational studies are needed to better define optimal TSH levels in the first trimester.

I also identified a worrying discrepancy between published guidelines and clinical practice. The reason for this is likely to be multi-factorial including a lack of familiarity amongst clinicians with the current guidelines [93, 274], high rates of unplanned pregnancies, non-compliance with levothyroxine [275], and inconsistencies in management strategies amongst endocrinologists and obstetricians [276]. Optimization of early gestation thyroid function in women on levothyroxine is however achievable. One approach is to increase the levothyroxine dose by two extra tablets a week on conception, representing an approximate dose increase of 30% [256]. This appears to be safe and effective, but requires wider dissemination of the guidance and a willingness on the part of individual women to independently adjust their doses. A second strategy [277] is to ensure that pre-conception TSH is maintained in the low-normal range (<1.2 mU/l) in order to increase the likelihood of optimal thyroid status in early gestation [277]. However close monitoring will be required to prevent over-treatment which was seen in only 5.1% of our sample based on a TSH <0.1 mU/l according to the ATA pregnancy guidelines)[93] Subclinical hyperthyroidism has not been shown to be harmful in pregnancy [278] and the small risk of its occurrence is largely

outweighed by the adverse effects of suboptimal replacement due to insufficient or late dose increases. Both low and high maternal FT4 levels may result in increased risk of lower offspring IQ which suggests that care must be to taken to correct low FT4 levels, but avoiding overtreatment is essential [76].

When compared to the risks of under-treatment, the benefits of levothyroxine optimization were substantial; 21 of the 49 (42.9%) miscarriages occurring at TSH levels >4.50 mU/l may have been prevented if they had the same rate of miscarriage as individuals with a TSH level between 0.2-2.5 mU/l. Thus my findings are also relevant to the current debate on universal thyroid screening. If levothyroxine therapy can reduce the risk of miscarriage in women with TSH levels above 4.5 mU/l to that observed in 0.2-2.5 mU/l, then there would be substantial gains at the population level from thyroid screening. However, clarifying treatment thresholds and implementing this policy would prove challenging. Furthermore, evidence from a large randomized controlled trial of correcting subclinical hypothyroidism and isolated hypothyroxinemia later in pregnancy (prior to 20 weeks) showed no benefit on obstetric outcomes[118]

The strengths of the this analysis are the use of a large well-validated population-based dataset with detailed clinical and biochemical data collected over a long period. The widespread use of electronic prescriptions by UK primary care physicians makes it unlikely that

individuals receiving levothyroxine were missed. Similarly, almost all laboratories in England were issuing electronic biochemical results by the year 2000 thus very few TSH results would have been excluded. Compared to studies based on hospital clinic records our dataset included data from a wide variety of practitioners and is therefore representative of the general population. In addition, I could identify early pregnancy losses which would have been missed in hospital based studies since most pregnant women do not enrol in hospital antenatal clinics until well into gestation. Indeed 15.3% of our identified miscarriages were the first entry of that pregnancy into the GPRD record and these events would certainly have been missed using hospital clinic records alone. Also this analysis is several times larger than previous studies I was able to identify a substantially greater number of miscarriages enabling us to better define risks according to TSH thresholds. Of practical importance is the longitudinal nature of our study which has highlighted the persistent nature of the problem in spite of published guidelines [268]. Finally, the use of observational data has allowed us to quantify the relative risks of over and under-treatment of gestational hypothyroidism which could not have been satisfactorily addressed in a non-observational study design.

Study limitations include the lack of data on some potential confounding factors most notably obstetric co-morbid conditions. Thus my observed associations between TSH levels and adverse obstetric outcomes could have been influenced by other undetermined obstetric factors. Furthermore, there be residual confounding from social class by linking

this to the patient's primary care practice rather than individual addresses which were unavailable to us. We also lacked data on free thyroxine levels and thyroid peroxidase antibody titres and therefore we were unable to clarify the impact of hypothyroxinaemia and thyroid autoimmunity on the outcomes observed in this study. We were unable to identify 17% of pregnancy outcomes which could have potentially led to ascertainment bias. It is however likely that the vast majority of unidentified outcomes result in normal deliveries, as adverse outcomes are more likely to be recorded. However if there was a differential recording of foetal loss by TSH level then this could have a substantial impact on effect estimates. However a sensitivity analysis assuming all unidentified outcomes were normal deliveries revealed similar results. Another key issue is that we didn't have data on women not on levothyroxine, I therefore could not assess whether results would be similar to those if screening on women with unknown thyroid status was undertaken during pregnancy. Nor could I assess differences between my study population and the "normal" pregnant population.

In summary, in this chapter I have demonstrated that the majority of levothyroxine treated women in this community-based cohort have early gestational TSH above the currently recommended targets. The best pregnancy outcomes were seen in women with target TSH levels and a strong risk of miscarriage was present at TSH levels exceeding 4.5 mU/l. There is therefore a pressing need for better liaison between endocrinologists and primary care practitioners to improve the adequacy

of thyroid hormone replacement in pregnancy or preferably before conception.

This analysis did not have any data on women who were not on levothyroxine, therefore other studies are needed to assess whether screening thyroid function is of benefit in pregnancy. This is the focus of my next chapter which uses trial data to ascertain if screening for and treating low thyroid function improves obstetric outcomes.

Chapter 7 Controlled Antenatal Thyroid Screening Study: Obstetric Outcomes

In the previous chapter, I highlighted that good control of thyroid function in pregnancy in women established on levothyroxine appeared to reduce the risk of miscarriage. In this chapter I will assess whether screening for and treating borderline low thyroid function during pregnancy results in improved obstetric outcomes. This chapter will combine data linkage to obtain obstetric outcomes with a large randomized controlled trial, the controlled antenatal thyroid screening study [116].

7.1 INTRODUCTION

It is well established that thyroid hormone is essential for an uncomplicated pregnancy and optimal foetal development [4]. Maternal thyroid hormone levels are especially important in the first half of pregnancy whilst the foetal thyroid is developing [4]. Thyroid disorders are also common in women of child-bearing age; furthermore, pregnancy increases demands on the hypothalamic-pituitary-thyroid axis[4]. As I have shown in the previous chapter in women established on levothyroxine, even modest variation in TSH levels was associated with increased risk of foetal loss.

At present, it is unclear if screening for and treating abnormal thyroid function is of benefit. Overt maternal hypothyroidism - elevated concentrations of TSH and low maternal free FT4 occurs in approximately 0.2-0.6% of pregnant women [4, 95, 96], whereas SCH (elevated TSH and normal FT4) can occur in up to 18% of pregnancies depending on the precise definition and TSH cut-point used [4, 93]. At present, all endocrine, thyroid, and obstetrical societies recommend initiating treatment for overt thyroid disease detected in pregnancy [85, 93, 269, 279, 280]. Although there is no consensus on screening for thyroid disease in pregnancy.

Given that SCH and isolated hypothyroxinemia (IH – FT4 below the 2.5 centile with TSH within the normal range) are more common in pregnancy than overt thyroid disease, it is important to quantify the risks they pose and assess the potential benefits of treatment if detected during pregnancy.

SCH is associated with similar adverse obstetric outcomes as overt hypothyroidism, albeit with a more modest effect [104]. Studies have demonstrated an increased incidence of adverse pregnancy outcomes with SCH including foetal loss, early pregnancy loss and admissions to the intensive care unit [106-110]. IH was originally considered to be a pregnancy specific condition possibly arising as a consequence of mild iodine deficiency. This concept has been more recently challenged as it occurs in iodine sufficient areas and does not typically resolve with iodine

supplementation [97, 98]. Other factors including elevated BMI, older age, iron status and placental angiogenic factors have all been identified as likely risk factors for IH [99-101].

Thyroid auto-immunity may also have a role as a meta-analysis of 19 observational cohort studies showed more than a tripling in the odds of miscarriage in the presence of thyroid antibodies OR=3.9, (95%CI: 2.48 to 6.12) [114]. This is an important observation as thyroid auto-immunity is a substantial risk factor for SCH [4] although there may be an important interactive effect with the combination of higher TSH levels and TPO antibody positivity resulting in substantially increased risk of adverse outcomes [93]. Furthermore, TPO antibody positivity may impair thyroidal response to hCG resulting in less responsiveness to the demands of pregnancy on the thyroid [272]. It is likely that thyroid hormone levels are not simply reflecting auto-immunity as levothyroxine in TPO antibody positive women may improve outcomes [273].

7.1.1 Treatment of SCH and IH detected during pregnancy

Two large randomized controlled trials the controlled antenatal thyroid study (CATS)[116] and a study by Casey et al. [118] have studied the effects of screening and treating borderline low thyroid function in pregnancy. The studies are summarized in **Table 47**. Neither study showed any beneficial effects of treatment on offspring IQ [116, 118]. Reasons for failure to establish treatment benefit include relatively late initiation of treatment (particularly in the Casey study) early age of IQ

assessment (particularly in the CATS study). Follow on analysis of the CATS study revealed no apparent benefit of treatment at age 9[129] although identified levothyroxine over-treatment may increase the risk of autism symptoms [281].

Table 47 Summary of the CATS and Casey randomized clinical trials

	CATS Study [116, 129]	Casey Study [118]
Countries	UK, Italy	USA
Number randomised	794	1203
Gestational age at recruitment (weeks)	<16	<21
Median TSH (mU/l)	3.8 (controls 3.2)	4.5 (controls 4.3)
Placebo-controlled	No	Yes
Offspring age at assessment (years)	3, 9	5

The original CATS study [116] did not collect detailed obstetric outcomes. Importantly, the CATS study was substantially earlier than in the Casey study[118]. This presents a unique opportunity to study the potential benefits on obstetric outcomes with earlier initiation of levothyroxine. Potential advantages include additional treatment time to influence adverse outcomes such as early gestational age. Other outcomes such as foetal loss, are less common in later pregnancy, and failure to detect treatment benefit here may reflect lack of power. Furthermore, the Casey study only assessed obstetric outcomes in those with abnormal thyroid function, the use of data-linkage enables those with normal

thyroid function to be studied as well. A further randomized control trial in this area would have substantial difficulties particularly as many clinicians now screen for and treat SCH, therefore repeating these studies would be challenging.

For this chapter I obtained detailed obstetric data from Welsh patients enrolled in the CATS study using data linkage in the Secure Anonymised Information linkage (SAIL) databank (described previously in the methods) [130]. This enabled me to assess the potential obstetric benefits of screening for sub-optimal maternal thyroid function in early pregnancy

7.2 METHODS

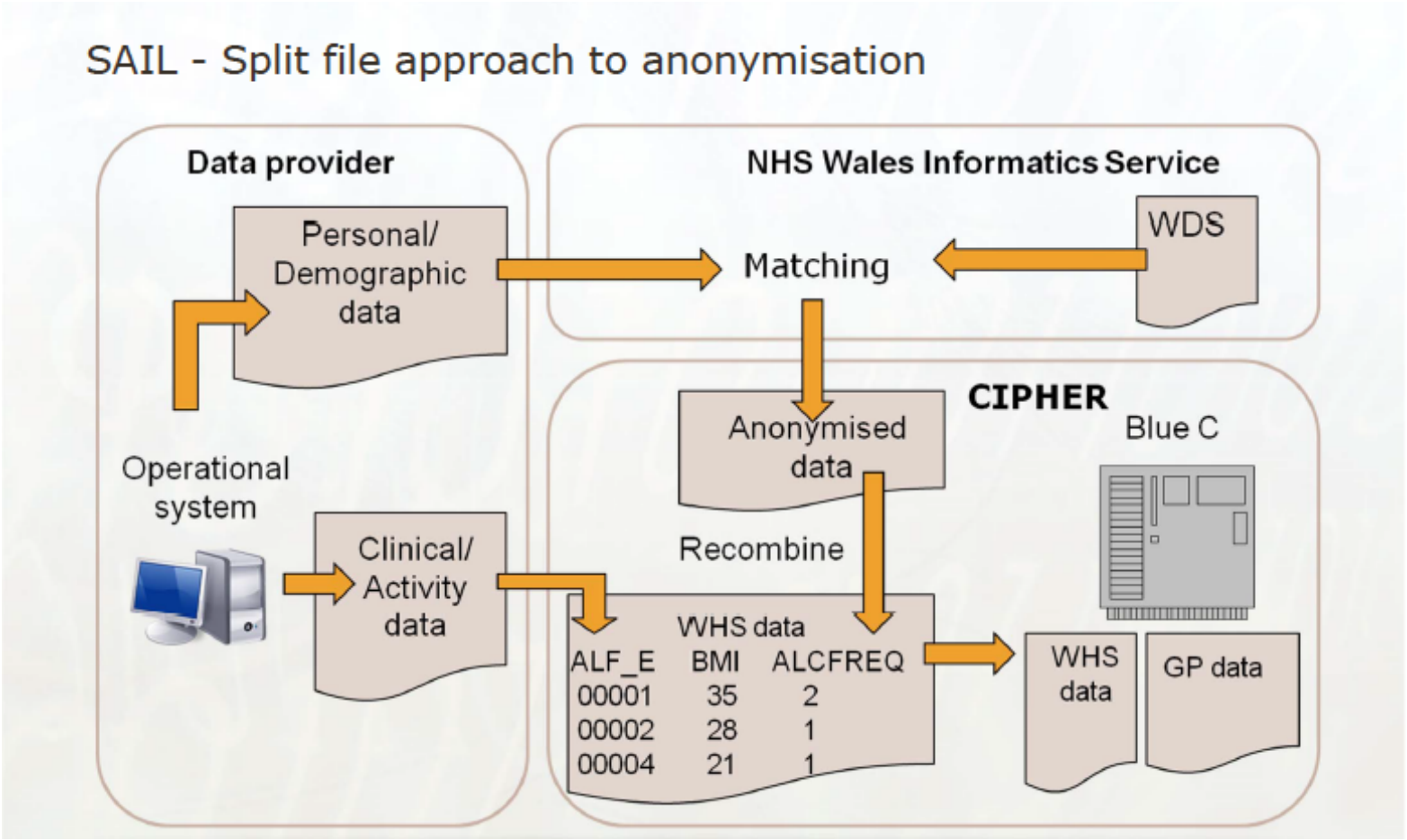
7.2.1 Study Cohort

The CATS study has been previously described in detail [116, 282] and already introduced in **Chapter 2**. In brief, CATS was a large randomized trial of 21,846 pregnant women recruited from the UK and Italy (15,752 from Wales). Pregnant women were recruited prior to 16 weeks gestation at which time 50% had TSH and FT_4 measured having been randomised to the screening group. The remainder (control group) had thyroid function measured after delivery. Women with TSH levels above the 97.5th percentile, and/or free T4 levels below the 2.5th percentile, were considered to have a positive screening result. Women with positive findings in the screening group received 150 mcg of levothyroxine per day with a median treatment initiation of 13 weeks 3 days. All patients

received 150mcg of levothyroxine. Thyroid function was performed 6 weeks later with a target TSH of 0.1 to 1.0 mU/l.

SAIL is a database of routinely collected health data run by the Health Informatics Research Unit (HIRU) at Swansea University. SAIL contains over two billion anonymised person-based records and is linked to other health and social care datasets including the Patient Episode Database for Wales (PEDW) [283]. PEDW is a register of all clinic and inpatient activity undertaken in Welsh NHS hospitals and processes over a million hospital episodes annually including information on diagnoses, admissions, hospital births, and surgical operations including Caesarean sections. Matching of SAIL data to CATS study data was undertaken using multiple approaches. These included the mother's name, date of birth, hospital number, home address during the CATS study and GP practice during the CATS study. The matching process in SAIL is shown in **Figure 20**.

Figure 20 Data linkage in SAIL



7.2.2 CATS data and SAIL obstetric data used in analyses

Data on maternal age, maternal weight, thyroid function (TSH and FT4), gestational age at recruitment, parity, trial group, whether levothyroxine treatment was initiated, and smoking history were all obtained from the CATS trial database with SAIL data providing obstetric outcomes. The pre-specified primary outcomes of interest were foetal loss, early gestational age (delivery before 37 weeks, and delivery before 34 weeks), need for Caesarean Section (overall) and early Caesarean section (before 37 weeks), whether the baby needed inducing, pre-eclampsia, low birth weight (<10^{th} centile) macrosomia (> 90^{th} centile) and an APGAR score <7 at 5 minutes.

7.2.3 Statistical analysis

All women recruited to the CATS study from the UK were eligible, however as congenital abnormalities will influence the risk of adverse obstetric outcomes independently of thyroid function these pregnancies were removed from the analysis.

Logistic regression was used to assess the odds of adverse outcomes. Analyses were performed to assess different groups 1) Those with abnormal thyroid function only – comparing those who were treated to those who were untreated. 2) Comparing those with normal thyroid function to those with abnormal thyroid function. 3) Comparing those with normal thyroid function to those who were untreated with abnormal thyroid function.

Analyses were adjusted for maternal age, maternal weight at recruitment, parity, smoking status, and sex of offspring. In analysis of just those with abnormal thyroid function analyses were also adjusted for maternal TSH and FT4 levels. Maternal age was available in all participants there was missing data in all other co-variates in the 14,376 individuals used in the final analysis: maternal weight at recruitment 1,162 (8.1%), parity 119 (0.83%), smoking status 777 (5.4%), sex of offspring, 119 (0.83%). Missing covariates were dealt with in adjusted models by multiple variable imputation.

Analyses were undertaken to compare outcomes with those with abnormal thyroid function versus those with normal thyroid function. Additionally, analyses were repeated by TSH level with TSH levels were categorized into <2.5 mU/l, 2.5-4.0 mU/l and greater than 4 mU/l in keeping with ATA thresholds [93]. Analyses were repeated with those who received treatment with levothyroxine removed to enable untreated individuals with low thyroid function to be compared to those with normal thyroid function.

7.2.4 Sensitivity analyses

As smoking during pregnancy is strongly associated with low birth weight and prematurity [284] this was used to confirm successful data linkage as smoking status was taken from the CATS study and these outcomes were taken from SAIL. Additionally, maternal weight is likely to be highly

correlated with offspring weight and this was also assessed as it used maternal weight from the CATS study and birthweight from SAIL.

There would also be a small number of women with undiagnosed hyperthyroidism. Therefore, to exclude individuals with hyperthyroidism from the normal group, women with hyperthyroidism TSH levels below 0.05 mU/l) and/or FT4 levels in the highest 2.5% (> 17.7 pmol/l) were also excluded in a sensitivity analysis.

Another issue was that a reasonably large dose of levothyroxine was given (150 mcg). In individuals with only borderline low thyroid function, this dose of levothyroxine resulted in FT4 levels being higher than 17.7pmol/l (97.5% centile of FT4 range in the UK CATS population) at subsequent blood tests in pregnancy. Analyses removing individuals who were over-replaced were also performed. Finally, to see if there was only benefit in early treatment we removed individuals started on levothyroxine after 14 weeks.

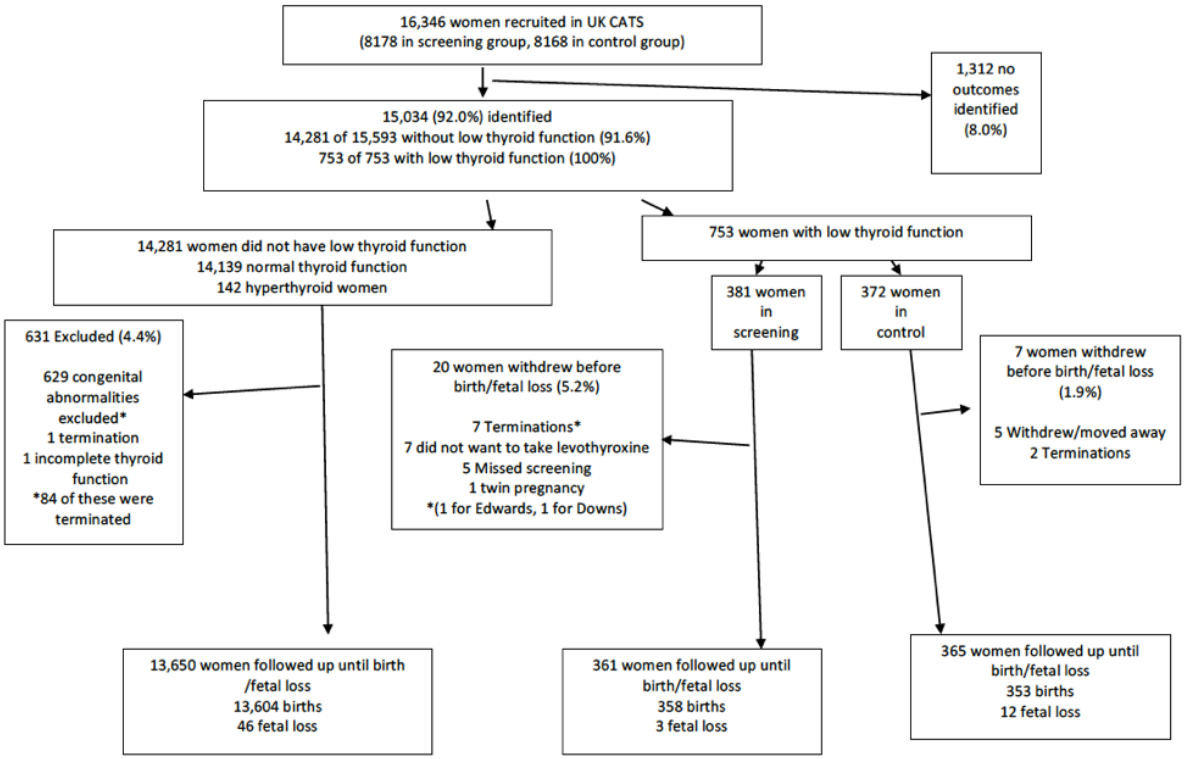
7.3 RESULTS

7.3.1 Study participant flow

In the 16,346 pregnancies from the UK in the original CATS study, birth outcome data were available in 15,034 (92.0%) pregnancies. In these there were 93 terminations and 61 foetal losses. 629 pregnancies were identified to have congenital abnormalities and these were excluded from analysis. Final outcome data were utilised in 13,650 women with

normal thyroid function and 726 women with abnormal thyroid function making 14,376 individuals used in total in the final analysis. Participant flow is summarized in **Figure 21**.

Figure 21 Participants of CATS study used in this analysis



7.3.2 TSH and FT4 Levels

As expected, TSH was not normally distributed with a median TSH of 1.12 mU/l (IQR 0.68 – 1.66 mU/l). The highest TSH recorded was 57.0 mU/l. Substantial numbers of women had modestly elevated TSH levels; 869 (6.03%) women had a TSH level between 2.5-4.0 mU/l, 246 women (1.71%) women had a TSH level between 4-10 mU/l and 17 women (0.12%) had a TSH level greater than 10mU/l.

FT4 was normally distributed (mean 14.0pmol/l SD 1.87pmol/l)) 142 women could be classed as hyperthyroid (TSH <0.05 mU/l and FT4 > 17.7 pmol/l). Of these 5 individuals had moderate thyrotoxicosis as evidenced by a FT4 > 30 pmol/l.

7.3.3 Summary of women with abnormal thyroid function

753 women had SCH and IH. Of these 726 were in the study until delivery or foetal loss of which 361 were treated and 365 were untreated. The reasons for the 27 women exiting the study before foetal loss or delivery are shown in **Figure 21**. More women left the screening group (n=20) vs the control group (n=7). In the treated group, there were 7 terminations, 7 people who did not want to take levothyroxine or attend for monitoring, 5 missed screenings (people had abnormal thyroid function, but were in error not identified as requiring levothyroxine) and 1 pregnancy which was found to be a twin pregnancy after recruitment, but before treatment was commenced and therefore excluded. In the control group,

there were 5 people who withdrew from the study and there were 2 terminations.

7.3.4 Comparison of baseline characteristics between the treated and untreated groups with abnormal thyroid function

As expected due to randomization, there was no clear difference between the treated and untreated groups with regard to maternal age, weight, previous pregnancy, smoking during pregnancy and sex off offspring (**Table 48**). However due to the nature of the original CATS where the threshold for treatment periodically changed to keep at the highest 2.5% for TSH and lowest 2.5% for FT4, TSH was higher at baseline in the treated group (3.76 mU/l) versus the untreated group (3.22 mU/l) although no significant difference was observed for FT4.

Table 48 Comparison of baseline characteristics in the treated and untreated groups (n=726)

Factor	Treated	Untreated	P value*
Maternal age (years)	30 (IQR 25-33)	30 (IQR 25-33)	0.94
Maternal weight (Kg)	69.9 (IQR 62-81.2)	68.5 (IQR 60.1 – 81.9)	0.50
% Previous pregnancy	51.6	52.7	0.79
% Smoking	20.5	18.7	0.58
% Male offspring	55.4	52.7	0.51
TSH level (mU/l)	3.76 (IQR 1.48 – 4.61)	3.22 (IQR 1.22-4.18)	0.003
FT4 level (pmol/l)	11.1 (IQR 10.5 – 13.1)	11.2 (IQR 10.4 – 13.2)	0.67

*Compared with the Wilcoxon rank sum test for continuous variables with non Gaussian distribution. The Chi Square test was used for categorical variables.

Comparison of obstetric outcomes between individuals in the treated group and the untreated group in those with abnormal thyroid function

A summary of TSH and FT4 and gestational age at foetal loss in women with abnormal thyroid function is shown in Table **49**. 10 miscarriages occurred in women with a TSH > 2.5 mU/l. In the treated group, foetal loss only occurred at more profound levels of hypothyroidism. Univariate analysis identified that women who did not receive levothyroxine had more foetal loss (12/365) than those who received treatment (3/361) OR=4.06 (95%CI 1.14, 15.5) p=0.03 which persisted after adjustment OR = 4.15 (95%CI 1.14, 15.2) p=0.03 **Table 50**.

Table 49 TSH and FT4 levels in women with abnormal thyroid function who had a foetal loss

Treated			Untreated		
TSH (mU/l)	FT4 (pmol/l)	Gestational age at foetal loss (weeks)	TSH (mU/l)	FT4 (pmol/l)	Gestational age at foetal loss (weeks)
9.37	13	< 24 weeks†	0.63	10.6	14.3
12.99	11.8	<24 weeks†	0.8	10.4	13.4
20.01	9.2	<18 weeks†	1.13	10.2	13.9
			1.38	9.9	25.3
			1.42	9.6	13.1
			2.16	10.1	27.8
			3.29	15.5	40.1
			3.32	14.5	25.4
			3.62	12.7	12.7
			3.66	13.8	38.7
			4.58	13.2	41.4
			4.73	13.9	-

†Exact gestational age at foetal loss unknown.

In the 711 live births, analyses were undertaken to see if treatment with levothyroxine improved other obstetric outcomes. No substantial benefits of levothyroxine were identified (**Table 50**). Untreated individuals did not have higher odds of delivery before 37 weeks OR=0.95 (95%CI 0.51, 1.79) p=0.88 or before 34 weeks OR=0.39 (95%CI 0.11, 1.36) p=0.14. Untreated individuals also did not have increased odds of requiring a Caesarean section OR=0.83 (95%CI 0.59, 1.17) p=0.28 or an

early Caesarean section (before 37 weeks) OR=1.03 (95%CI 0.42, 2.55) p=0.94 or having labour induced OR =1.29 (95%CI 0.93, 1.80) p=0.12. There was also no difference in odds in untreated individuals for offspring outcomes including low birth weight OR = 1.39 (95%CI 0.77, 2.51) p=0.28, macrosomia OR=1.03 (95%CI 0.58, 1.82) p=0.92 and APGAR score <7 OR=1.21 (95%CI 0.10, 15.6) p=0.88.

Table 50 Obstetric outcomes in individuals with abnormal thyroid function by treatment status

Outcome	Treated		Untreated				Untreated #		
	N	OR	N	OR	95%CI	p	OR	95%CI	p
Still birth	3/358	1	12/353	4.06	1.14, 14.5	0.03	4.15	1.14, 15.1	0.03
711 live births									
Delivery <37 week[1]	22/357	1	21/352	0.97	0.52, 1.79	0.91	0.95	0.51, 1.79	0.88
Delivery <34 week[1]	9/357	1	4/352	0.44	0.14, 1.46	0.18	0.39	0.11, 1.36	0.14
Caesarean Section[2]	98/355	1	86/353	0.84	0.60, 1.18	0.33	0.83	0.59, 1.17	0.28
Caesarean Section[2] <37 weeks	10/355	1	11/353	1.11	0.47, 2.65	0.82	1.03	0.42, 2.55	0.94
Induced[3]	235/352	1	252/349	1.29	0.94, 1.79	0.12	1.29	0.93, 1.80	0.12
Pre-eclampsia									
Birth weight centile <10[4]	23/295	1	32/303	1.40	0.80, 2.85	0.24	1.39	0.77, 2.51	0.28
Birth weight centile >90[4]	29/295	1	30/303	1.01	0.59, 1.73	0.98	1.03	0.58, 1.82	0.92
Apgar <7 at 5 mins[5]	1/265	1	2/265	2.01	0.18, 22.3	0.57	1.21	0.10, 15.6	0.88

[1] 2 individuals with missing data
[2] 3 individuals with missing data
[3] 10 individuals with missing data
[4] 113 individuals with missing data
[5] 181 individuals with missing data
# Adjusted for maternal age, maternal weight at recruitment, parity, smoking status, sex of offspring, TSH level, FT4 level
N=number. OR = odds ratio, CI = confidence interval p = p value against the null hypothesis of no association.

7.3.5 Comparing outcomes women with abnormal (low) thyroid function to the rest of the cohort

Women older than 30 years OR = 1.28 (95%CI 1.10, 1.49) p=0.001 and women heavier than 90 Kg OR =1.51 (95%CI 1.21, 1.88) p<0.001 were more likely to have abnormal thyroid function. No substantial difference in odds of abnormal thyroid function was observed for smokers OR =0.87 (95%CI 0.71, 1.08) p=0.20, foetal female sex OR =0.88 (95%CI 0.75, 1.04) p=0.13 or previous childbirth OR =0.95 (95%CI 0.80, 1.12) p=0.55.

Women with abnormal thyroid function were more likely to suffer from foetal loss than those with normal thyroid function OR =5.85 (95%CI 3.24, 10.6) p<0.001. No clear difference was observed for any other obstetric outcomes (**Table 51**).

Table 51 Comparing outcomes in women with normal thyroid function to those with abnormal thyroid function

Outcome	Normal N	Effect	Abnormal N	OR	95%CI	p	Abnormal # OR	95%CI	p
Foetal loss 14,315 live births	46/13,650	1	15/726	6.23	3.46, 11.2	<0.001	5.85	3.24, 10.6	<0.001
<37 weeks[1]	763/13,604	1	43/709	1.09	0.79, 1.49	0.61	1.08	0.79, 1.49	0.63
<34 weeks[1]	201/13,604	1	13/709	1.25	0.71, 2.19	0.45	1.26	0.71, 2.22	0.43
Caesarean Section[2]	3,181/13,182	1	184/708	1.10	0.93, 1.31	0.26	1.02	0.85, 1.21	0.81
Caesarean Section <37 weeks[2]	269/13,182	1	21/708	1.47	0.94, 2.30	0.10	1.42	0.91, 2.23	0.13
Pre-eclampsia[3]	571/13,178	1	21/567	0.82	0.52, 1.27	0.38	0.74	0.47, 1.16	0.19
Birth weight centile <10[4]	1395/13,485	1	55/598	0.88	0.66, 1.16	0.37	0.94	0.70, 1.25	0.67
Birth weight centile >90[4]	1047/13,485	1	59/598	1.30	0.99, 1.71	0.06	1.19	0.90, 1.58	0.22
Apgar <7 at 5 mins[5]	105/12,142	1	3/530	0.65	0.21, 2.06	0.47	0.62	0.19, 1.97	0.42

1 2 people with missing data
2 425 people with missing data
3 545 people with missing data
4 232 people with missing data
5 1,643 people with missing data
N=number. OR = odds ratio, CI = confidence interval p = p value against the null hypothesis of no association
# Adjusted for maternal age, maternal weight at recruitment, parity, smoking status, sex of offspring

Removing individuals who received treatment revealed no substantial differences in obstetric outcomes, aside from foetal loss. The effect estimate was higher OR=9.61 (95%CI 5.03, 18.4) p<0.001 (**Table 52**).

Analyses of odds of adverse outcomes by TSH thresholds as recommended by the American Thyroid Association[93] were performed. Removing individuals who received treatment, higher TSH levels were associated with increased odds of fetal loss, but this was only apparent at TSH levels >4.0 mU/l - OR=5.85 (95%CI 1.29, 26.5) but no substantial differences were observed for other obstetric outcomes **(Table 53**). Including individuals who were treated did attenuate the association with higher TSH and fetal loss OR= 4.82 (95%CI 1.90, 12.2) but had no apparent effect on other obstetric outcomes (**Table 54**).

Table 52 Comparing obstetric outcomes in women with normal thyroid function to those with abnormal thyroid function who were untreated

Outcome	Normal N	Effect	Abnormal N	OR	95%CI	p	Abnormal # OR	95%CI	P
Fetal loss	46/13,650	1	12/365	10.1	5.27, 19.1	<0.001	9.61	5.03, 18.4	<0.001
13,957 live births									
Delivery <37 week[1]	763/13,604	1	21/352	1.07	0.68, 1.67	0.77	1.07	0.69, 1.69	0.76
Delivery <34 week[1]	201/13,604	1	4/352	0.77	0.28, 2.07	0.60	0.78	0.28, 2.12	0.63
Caesarean Section[2]	3,181/13,182	1	86/353	1.01	0.79, 1.30	0.92	0.95	0.74, 1.22	0.67
Caesarean Section <37 weeks[2]	269/13,182	1	11/353	1.54	0.84, 2.85	0.16	1.52	0.83, 2.81	0.18
Pre-eclampsia[3]	571/13,178	1	10/298	0.77	0.41, 1.45	0.41	0.71	0.38, 1.35	0.30
Birth weight centile <10[4]	1395/13,485	1	32/303	1.02	0.71, 1.48	0.90	1.09	0.75, 1.60	0.64
Birth weight centile >90[4]	1047/13,485	1	30/303	1.31	0.89, 1.91	0.17	1.22	0.82, 1.80	0.33
Apgar <7 at 5 mins[5]	105/12,142	1	2/265	0.87	0.21, 3.55	0.85	0.85	0.21, 3.46	0.82

Adjusted for maternal age, maternal weight at recruitment, parity, smoking status, sex of offspring,
1 1 person with missing data
2 422 people with missing data
3 477 people with missing data
4 169 people with missing data
5 1,550 people with missing data

Table 53 Comparison of obstetric outcomes by ATA TSH thresholds with treated individuals excluded

Outcome	N	TSH Level (mU/l)	TSH Level OR	95%CI	p	TSH level # OR	95%CI	p
Foetal loss	52/13,094	<2.5	1					
	4/757	2.5-4	1.22	0.44, 3.39	0.06	1.16	0.38, 3.51	0.12
	2/106	>4	6.88	1.65, 28.8		5.85	1.29 26.5	
13,957 live births								
Birth <37 weeks[1]	739/13,094	<2.5	1			1		
	37/756	2.5-4	0.86	0.61, 1.21	0.86	0.86	0.61, 1.20	0.86
	8/106	>4	1.36	0.66, 2.82		1.38	0.67, 2.86	
Birth < 34 weeks[1]	194/13,094	<2.5	1			1		
	10/756	2.5-4	0.89	0.47, 1.69	0.58	0.91	0.48, 1.73	0.65
	1/106	>4	0.63	0.09, 4.56		0.67	0.09, 4.86	
Caesarean Section[2]	3,035/12,692	<2.5	1					
	211/737	2.5-4	1.28	1.08, 1.51	0.09	1.18	1.00, 1.39	0.56
	21/106	>4	0.77	0.49, 1.27		0.69	0.42, 1.11	
Caesarean Section<37 weeks[2]	259/12,692	<2.5	1					
	17/737	2.5-4	1.13	0.69, 1.86	0.26	1.12	0.68, 1.85	0.27
	4/106	>4	1.88	0.69, 5.15		1.88	0.69, 5.17	
Pre-eclampsia[3]	550/12,118	<2.5	1			1		
	29/720	2.5-4	0.92	0.63, 1.35	0.36	0.80	0.54, 1.17	0.11
	2/92	>4	0.49	0.12, 1.99		0.43	0.11, 1.77	
Birth Weight Centile <10[4]	1,330/12,954	<2.5	1			1		
	88/741	2.5-4	1.17	0.94, 1.48	0.30	1.26	1.00, 1.54	0.08
	9/93	>4	0.94	0.47, 1.87		1.08	0.54, 2.19	
Birth Weight Centile >90[4]	1,003/12,954	<2.5	1			1		
	65/741	2.5-4	1.15	0.88, 1.49	0.22	1.10	0.84, 1.44	0.47
	9/93	>4	1.28	0.64, 2.55		1.11	0.55, 2.25	

Adjusted for maternal age, maternal weight at recruitment, parity, smoking status, sex of offspring,
1 1 person with missing data 2 422 people with missing data,3 477 people with missing data 4 169 people with missing data

Table 54 Comparison of obstetric outcomes by ATA TSH thresholds

Outcome		TSH Level				TSH level #		
	N	TSH Level (mU/l)	OR	95%CI	p	OR	95%CI	p
Foetal loss	52/13,234	<2.5	1			1		
	4/834	2.5-4	1.18	0.43, 3.28	0.002	1.14	0.41, 3.18	0.002
	5/247	>4	5.15	2.04, 13.0		4.82	1.90, 12.2	
14,315 live births								
Birth <37 weeks[1]	747/13,233	<2.5	1			1		
	43/833	2.5-4	0.91	0.66, 1.25	0.97	0.91	0.66, 1.24	0.98
	16/247	>4	1.16	0.69, 1.93		1.16	0.69, 1.93	
Birth < 34 weeks[1]	199/13,233	<2.5	1			1		
	12/833	2.5-4	0.96	0.53, 1.72	0.71	0.98	0.54, 1.75	0.79
	3/247	>4	0.81	0.26, 2.53		0.84	0.27, 2.65	
Caesarean Section[2]	3,075/12,831	<2.5	1			1		
	234/814	2.5-4	1.28	1.09, 1.50	0.09	1.18	1.00, 1.38	0.66
	56/245	>4	0.94	0.70, 1.27		0.83	0.61, 1.13	
Caesarean Section<37 weeks[2]	263/12,831	<2.5	1			1		
	19/814	2.5-4	1.14	0.71, 1.83	0.18	1.12	0.70, 1.80	0.20
	8/245	>4	1.61	0.79, 3.30		1.61	0.78, 3.29	
Pre-eclampsia[3]	551/12,774	<2.5	1			1		
	33/784	2.5-4	0.97	0.68, 1.40	0.71	0.84	0.58, 1.20	0.24
	8/212	>4	0.87	0.43, 1.77		0.76	0.37, 1.57	
Birth Weight Centile <10[4]	1,337/13,062	<2.5	1			1		
	92/805	2.5-4	1.13	0.90, 1.42	0.58	1.22	0.97, 1.54	0.18
	21/216	>4	0.94	0.60, 1.49		1.06	0.67, 1.68	
Birth Weight Centile >90[4]	1,012, 13,062	<2.5	1			1		
	73/805	2.5-4	1.19	0.93, 1.52	0.09	1.12	0.87, 1.44	0.27
	21/206	>4	1.28	0.81, 2.02		1.18	0.74, 1.88	

Adjusted for maternal age, maternal weight at recruitment, parity, smoking status, sex of offspring,
1 2 people with missing data, 2 425 people with missing data

7.3.6 Sensitivity analyses

Sensitivity analysis – matching

Data matching appears to be robust. Smokers were more likely to have children with low birthweight (less than 10th centile) than non-smokers OR=2.70 (95%CI 2.40, 3.04) p=<0.001. Smokers were also more likely to have children born before 37 weeks OR=1.44 (95%CI 1.23, 1.70) p<0.001 and before 34 weeks OR =1.86 (95%CI 1.41, 2.46) p<0.001. Variation in maternal weight in pregnancy explained 56% of the variation in offspring birthweight and was strongly associated with it (p<0.001).

Sensitivity analysis: Removing women who were over-treated from the analysis

As a substantial dose of levothyroxine was given to all individuals, 97 of the 358 women who were treated were identified as being over-replaced with a subsequent FT4 greater than 17.7 pmol/l. Given over-treatment might negate some of the treatment benefits analyses were repeated excluding these 97 individuals. Removing these individuals did not appear to have a substantial effect on effect estimates. (**Table 55**).

Sensitivity analysis: Removing women who were commenced on treatment after 13 weeks

117 women were started on levothyroxine after 13 weeks. They were removed from analysis. No substantial effect was seen on obstetric outcomes (**Table 56**) indicating no additional benefit at treating at a slightly earlier time point.

Table 55 Obstetric outcomes in women with abnormal thyroid function with over-treated individuals removed from analysis

Outcome	Treated		Untreated				Untreated #		
	N	Effect	N	OR	95%CI	P	OR	95%CI	p
Still birth 614 live births	3/261	1	12/353	2.96	0.83, 10.6	0.10	2.91	0.78, 10.8	0.11
Odds <37 week[1]	16/260	1	21/352	0.97	0.49, 1.83	0.92	0.93	0.46, 1.87	0.84
Odds <34 week[1]	9/260	1	4/352	0.32	0.10, 1.05	0.06	0.28	0.08, 0.99	0.05
Caesarean Section[2]	70/258	1	86/353	0.85	0.59, 1.22	0.39	0.85	0.58, 1.25	0.42
Caesarean Section <37 weeks[2]	7/258	1	11/353	1.16	0.44, 3.03	0.77	1.11	0.40, 3.04	0.84
Induced[3]	173/255	1	252/349	1.23	0.87, 1.75	0.25	1.23	0.86,1.77	0.26
Pre-eclampsia[4]	7/195	1	10/298	1.10	0.43, 2.85	0.84	1.26	0.46, 3.40	0.65
Birth weight centile <10[5]	13/199	1	32/303	1.70	0.87, 3.32	0.12	1.58	0.77, 3.22	0.22
Birth weight centile >90[5]	20/199	1	30/303	0.99	0.54, 1.80	0.97	1.17	0.62, 2.22	0.63
Apgar <7 at 5 mins[6]	1/183	1	2/265	1.39	0.13, 15.5	0.79	-	-	-

Adjusted for maternal age, maternal weight at recruitment, parity, smoking status, sex of offspring, TSH and FT4
1 2 individuals with missing data
2 3 individuals with missing data
3 10 individuals with missing data
4 130 individuals with missing data
5 112 individuals with missing data
6 166 individuals with missing data
APGAR <7 at 5 mins could not be calculated in adjusted models due to colinearity

Table 56 Obstetric outcomes in women with abnormal thyroid function and removing all individuals who started treatment after 13 weeks

Outcome	Treated		Untreated				Untreated #		
	N	Effect	N	OR	95%CI	p	OR	95%CI	p
Still birth	3/255	1	12/365	2.86	0.80, 10.2	0.11	2.87	0.78, 10.5	0.11
602 live births									
Odds <37 week[1]	19/247	1	22/353	0.80	0.42, 1.51	0.49	0.83	0.43, 1.60	0.58
Odds <34 week[1]	7/247	1	5/353	0.49	0.15, 1.57	0.23	0.55	0.16, 1.82	0.33
Caesarean Section[2]	69/246	1	86/353	0.83	0.57, 1.20	0.31	0.85	0.58, 1.24	0.39
Caesarean Section[2] <37 weeks	9/246	1	11/353	0.85	0.35, 2.08	0.72	0.91	0.36, 2.30	0.83
Induced[3]	165/245	1	252/349	1.26	0.88, 1.80	0.20	1.23	0.84, 1.80	0.30
Pre-eclampsia[4]	10/199	1	10/298	0.66	0.27, 1.61	0.36	0.69	0.27, 1.77	0.44
Birth weight centile <10[5]	16/204	1	32/303	1.39	0.74, 2.60	0.31	1.36	0.70, 2.65	0.36
Birth weight centile >90[5]	20/204	1	30/303	1.01	0.56, 1.83	0.97	1.11	0.59, 2.11	0.74
Apgar <7 at 5 mins[6]	1/189	1	2/265	1.43	0.13, 15.9	0.77			

Adjusted for maternal age, maternal weight at recruitment, parity, smoking status, sex of offspring TSH and FT4 level
1 2 people with missing data
2 3 people with missing data
3 8 people with missing data
4 105 people with missing data
5 95 people with missing data
6 148 people with missing data

Screening of women to determine if they had abnormal thyroid function

Screening for women on the basis of age and weight may be practical in that data are readily available to healthcare practitioners. Screening women aged greater than 30 years would involve screening 46.5% of the population and would detect 52.5% of those with abnormal thyroid results. Increasing the age to greater than 35 years would results in 12.2 % of the population being screened and 14.1% of those with abnormal thyroid function being detected. Screening women with a weight greater than 90 kg would involve screening 9.74% of the population and detect 13.8% of those with abnormal thyroid function.

7.4 DISCUSSION

My results show that sub-optimal maternal thyroid function is associated with increased odds of foetal loss compared to women with normal thyroid function. This effect is reduced in women who receive levothyroxine who had a fourfold reduction (3 vs 12) in foetal loss compared to those who were untreated. Women who were untreated had a tenfold increased risk of foetal loss compared to those with normal thyroid function. Screening and treating for low thyroid function could potentially reduce 18 of the 64 foetal losses identified in the study (28.1%). Whilst the number needed to screen to potentially prevent 1 miscarriage is quite high - 18 foetal losses potentially prevented by screening 15,034 women; giving a number needed to screen of 835 to prevent 1 foetal loss. The number needed to treat is substantially lower at 40 women to be treated to prevent 1 foetal loss. This finding would lend strong support to implementing universal thyroid screening in pregnancy with the aim of reducing foetal loss.

My results are at first inspection are in conflict with a recent large randomized controlled trial by Casey at al. which used a similar study design, but found no benefit in terms of obstetric outcomes [118]. Two key differences in the trial and its analysis are noteworthy. The Casey trial recruited later in pregnancy and the Casey trial analysed SCH and IH separately, whereas in the original CATS study, both were analysed together [116]. Intriguingly in the Casey study there were more foetal losses in women with abnormal thyroid function who did not receive

levothyroxine - there were 18 foetal losses in total with 6 foetal losses in the treated group, and 12 in the untreated group [117]. As in the Casey study we observed no benefit of levothyroxine therapy with other obstetric outcomes, however it remains to be seen if initiating levothyroxine even earlier than the CATS study might improve other obstetric outcomes. Although our observation that neither SCH or IH were associated with substantially earlier gestational age is in keeping with a recent meta-analysis [285].

My foetal loss data however, is of potentially substantial importance to universal thyroid screening in pregnancy a key debate in thyroidology. The criteria for undertaking screening programs as laid down by Wilson and Jungner is shown in the box on the next page.

Considerations when deciding on implementing screening

1. Is the condition an important health problem?
2. Does the condition have an accepted treatment?
3. Are facilities for both diagnosis and treatment readily available ?.
4. Is there a recognizable latent or asymptomatic stage?
5. Is there a suitable screening test or examination?
6. Is the screening test acceptable to the population?
7. Is the natural history of the condition, including development from latent to declared disease adequately understood?
8. Is there an agreed policy on whom to treat ?
9. Is the cost of case-finding (including diagnosis and treatment of patients diagnosed) economically viable?
10. Case-finding should be a continuing process.

Adapted from Wilson J, Jungner G: Principles and practice of screening for disease. Geneva: World Health Organization, 1968. *Public health papers* 34, (2011)[286]

It is already clear that thyroid dysfunction is an important health problem (criteria 1), resulting in adverse obstetric and offspring outcomes [4]. This still holds true even if only overt hypothyroidism and overt hyperthyroidism are considered. However, this analysis has indicated potential benefits of treating borderline thyroid function. Furthermore, almost 2% of women had a TSH level above 4.0 mU/l and over 8% had a TSH level greater than 2.5 mU/l. Treatment of both overt

hypothyroidism and hyperthyroidism results in improved outcomes and is cost-effective and acceptable to patients (criteria 2), however the TSH and FT4 threshold for initiation of levothyroxine during pregnancy is less clear. Data from this work has indicated benefits at modest TSH and FT4 levels.

Facilities for both diagnosis and treatment are readily available (criteria 3) and there is also a well-recognized asymptomatic stage (criteria 4). Clinical assessment of thyroid status and thyroid function testing are both commonplace and are already readily acceptable to the general population (criteria 5 and 6). The natural history of subclinical thyroid dysfunction leading to overt hypothyroidism/hyperthyroidism is well understood, however it needs highlighting that many women with subclinical hypothyroidism would not progress to overt hypothyroidism if left untreated (criteria 7). The costs of case finding are also economically balanced even if only overt thyroid disease is considered [287] (criteria 9). The nature of thyroid screening in pregnancy ensures a continuing process and not a "once and for all" project (criteria 10).

Realistically only criteria 8 "there should be an agreed policy on whom to treat as patients" is not satisfied as more data on the benefits of levothyroxine therapy in women with subclinical hypothyroidism, isolated hypothyroxinaemia, and euthyroid autoimmunity are needed. Given there is widespread variation in current practice at present, this criteria alone should not prevent the implementation of universal

screening. Furthermore, this data largely supports current ATA TSH thresholds for initiation of levothyroxine in pregnancy [93]. A key feature of this guidance is that levothyroxine treatment should definitely be initiated at TSH levels greater than 10mU/l if TPO antibody negative and at greater than 4.0 mU/l if TPO antibody positive. The guidance also proposes that levothyroxine treatment can be considered at TSH levels greater than 2.5 mU/l if TPO antibody positive. TPO antibody levels were unfortunately not measured routinely in CATS, however I observed that greater risk of foetal loss became apparent at TSH levels greater 3.0mU/l with a substantial increase above 4.0 mU/l. Thus, it appears current ATA guidance is broadly correct (**Table 52)**.

These considerations notwithstanding, we still found that some women with profoundly elevated TSH had normal obstetric outcomes, a finding which has previously been reported [288]. Women found to have profoundly abnormal thyroid function during pregnancy can be reassured that obstetric outcomes do appear to be reasonable, particularly if treated.

The strength of this study is the linkage of a large and unique randomized controlled trial in pregnancy with multiple obstetric outcomes through a nationwide linked database. The high capture rate (92.9%) of pregnancy outcomes and expected findings from smoking confirms the robust data linkage in our study. Furthermore, we have used obstetric outcomes that are of key importance. One limitation of our study is that we were unable

to include the entire CATS cohort as we could not identify outcomes for all women in the CATS study. Adverse outcomes were more identifiable in those with abnormal thyroid function as they were more closely followed up (100% capture). There were also more terminations in the screening group versus the control group (7 vs 2) although the reason for this discrepancy is unclear and may be due to random error. There was a higher withdrawal rate in the treated group, but this is largely due to more terminations and that some women refused to take levothyroxine. Owing to the original design of the study, which had changes in TSH thresholds over the course of the study women in the treated group were more likely to have a higher TSH at baseline however this would likely bias our results towards the null. The number of statistical tests performed in this analysis, especially including the sensitivity analyses was substantial and has increased the risk of type-1 error.

The use of routinely collected data raises additional issues as routinely collected data tends to have high positive predictive values but may have lower sensitivity. However obstetric outcomes are well recorded on patient episode statistics as hospitals are incentivised to return accurate and routinely validated records. Although the use of this approach may be less robust than in the data collection of highly focused clinical trials it has enabled us to also explore outcomes in those with normal thyroid function.

Overall my results are supportive for identifying and correcting low thyroid function in pregnancy given the substantial benefits of reducing the risk of foetal loss. Replication is necessary, particularly using lower initial levothyroxine doses and in iodine sufficient areas. The use of a high levothyroxine doses does not appear to have been harmful in terms of obstetric outcomes, but however may have adverse effects on offspring. Recent data from a prospective population based pregnancy cohort, the Generation R study, indicates that the relationship between maternal FT4 levels and IQ is U shaped and therefore high or high-normal FT4 levels may potentially negatively impact on offspring neurological development [76] and follow-up data from the CATS study has indicated that higher ADHD scores were recorded in offspring in women who were untreated. Therefore more cautious levothyroxine dosing is required in pregnancy to offset against potentially deleterious effects on offspring IQ. Clarification is also needed as to whether substantial additional benefits might be obtained with preconception or even earlier pregnancy levothyroxine initiation and this should be the focus of future studies.

Chapter 8 Thesis Discussion

In this thesis, I have explored the relationship between common variation in thyroid status and adult health. I have demonstrated that even modest variation in thyroid status is associated with a key range of health outcomes. Pregnancy in particular, places additional demands on the thyroid and is a critical period, where even borderline low thyroid function is associated with an increased risk of foetal loss.

I have also observed the HPT axis is more complex than previously envisaged, with FT3 being less regulated by the HPT axis than FT4 and influenced by external factors including body composition and pubertal status. FT3 is also more variable over childhood and children appear to have different thyroid reference ranges to adults with a substantial proportion of children at age 7 having a FT3 above the adult reference range. FT3 may also be a reflection of nutritional status pre-puberty rather than simply being a mediator of thyroid status. We also appear to frequently modify the HPT axis in adults by routinely treating fairly modest low thyroid function with a high likelihood of subsequent over-treatment, and many individuals are also undertreated, which is of particular relevance in women of child-bearing age.

Therefore, two clear important themes from this thesis emerge. The first theme is that I have shown that even modest variation thyroid status is a *modifiable* risk factor for adverse outcomes. This was demonstrated in pregnancy where I have demonstrated that screening and treating for low

thyroid function is associated with reduced odds of foetal loss and women established on levothyroxine also have lower odds of foetal loss if well controlled. Preliminary data from the follow up to the CATS study, has also demonstrated that thyroxine treatment in pregnancy results in a reduction in maternal weight gain post-pregnancy indicating maternal as well as offspring benefits. The second theme is confirmation that assessment of thyroid function status is more complex than anticipated with serum FT3 likely being a poor marker of intracellular T3 status.

To extend the first theme, we see that variation of TSH within the population reference range is associated with a range of key health outcomes, although both higher and lower levels are associated with adverse outcomes. This U shaped curve indicates there may be an optimal zone of thyroid status as observed for instance with maternal FT4 and offspring IQ [289]. Earlier screening and intervention in pregnancy may produce even more profound benefits on foetal loss, and should assess whether it may influence other pregnancy outcomes including gestational age at delivery and low birth weight.

My pregnancy findings may also provide insight into variation in thyroid status in the general adult population. Whilst pregnancy may be a particularly critical time for optimal thyroid levels the sudden increased demands on the thyroid may cause a substantial change in intracellular thyroid levels especially in TPO positive individuals. It may be that tissues adapt to their prevailing thyroid function, and respond poorly to changes.

If there is an adaptation to a prevailing thyroid state minor variation within the reference range, can have important health consequences for an individual as it is a level of thyroid function their tissues are not prepared for.

For instance, treatment of borderline low thyroid function in adults younger than 65 years may reduce adverse cardiovascular outcomes [191]. The recent thyroid hormone therapy trial for older adults with subclinical hypothyroidism did not find benefits of correcting low thyroid function on mood or tiredness but was underpowered for cardiovascular outcomes [249]. Prospective trials of younger adults with subclinical hypothyroidism with a longer follow-up to properly explore cardiovascular outcomes are still urgently needed.

Given the potential importance of even minor variation in thyroid status data shows that our management of hypothyroidism in adults and particularly in women of child-bearing age could be substantially improved. Widespread thyroid function testing has resulted in few individuals with overt thyroid disease being undiagnosed [30]. However, the current UK approach to managing hypothyroidism results in many individuals having borderline low thyroid function corrected, but due to inadequate monitoring, many of them are converted to borderline high thyroid function whilst some remain inadequately treated. At 5 years post levothyroxine initiation 16% of patients had a TSH less than 0.5 mU/l and 21.5% had a TSH greater than 5.0 mU/l. The risk benefit of this practice

is unclear, and is likely to vary between body systems, but as we have observed in pregnancy modest differences may have substantial adverse outcomes, with prolonged sub-optimal treatment. Our widespread over-treatment of young women could substantially increase the risk of osteoporosis. Overtreatment is associated with increased fracture risk and adverse cardiovascular outcomes in small cohorts [246].

The second theme to emerge is that assessment of thyroid function status is more complex than anticipated. Our data from epidemiological cohorts and in pregnancy shows that high TSH and low FT4, which might be expected to have similar outcomes as both indicate low thyroid function, in fact have separate and distinct outcomes. This is shown most clearly in pregnancy where SCH and IH are associated with distinct and disparate outcomes [4]. Recent ATA guidance [93] also modifies the recommend TSH level for intervention with levothyroxine based on TPO antibody status. This reflects that TSH does not capture all of the variation in thyroid status with regard to adverse outcomes. The differing relationship of FT3 and FT4 to TSH over childhood also demonstrates the HPT axis is not as straightforward as we think and FT3 is more fluid. FT3 although a reliable sign of thyrotoxicosis is not a reliable marker of hypothyroidism and is likely a poor indicator of tissue FT3 [2].

As a result, an alternative means of assessing thyroid status may be desirable as while serum TSH, FT3 and FT4 also do not fully incorporate the extent of peripheral and intracellular regulation. An extreme

example of this is reflected in Allan-Herndon-Dudley syndrome which is a rare disorder of brain development that causes moderate to severe intellectual disability due to intracellular hypothyroidism from failure of MCT8 receptors to transport T3 into nerve cells effectively [290]. Excess amounts of T3 circulate in the bloodstream resulting in some tissues such as the liver and heart becoming hyperthyroid. Peripheral regulation of thyroid status may be more important when considering health outcomes than previously realised. The deiodinases, particularly DIO2 which converts intracellular FT4 to FT3 [291] may have a more substantial impact on intracellular FT3 than serum FT3 levels. This may be a key issue for the brain in particular as animal studies have indicated that serum tri-iodothyronine (T_3) contributes just 20% of intracellular T_3 in the cerebral cortex, the remainder coming from local deiodination of serum thyroxine (T_4) by deiodonase-2 [291, 292]. Genetic variation in the deiodinases have been associated with a wide range of phenotypes including patient satisfaction with thyroid hormone replacement [187].

Taken together, there is a compelling case for a better tissue specific marker for thyroid status NMR tissue spectroscopy would be on option which has considerable potential for non-invasive characterisation of tissue biochemistry and the diagnosis of tissue abnormalities. Another approach is to study metabolomics – the systematic study of the unique chemical fingerprints that specific cellular processes leave behind. Given the importance of thyroid hormone on intracellular processes it is highly likely metabolic signatures of intracellular hypothyroidism and

hyperthyroidism or “thyroid stress” can be identified. Metabolomic differences within the population reference range of thyroid status have already been identified [293]. Serum FT4 concentrations are strongly linked to serum acylcarnitines and phosphatidylcholines, indicating enhanced transport of fatty acids to mitochondrion and subsequent ß-oxidation [293]. Metabolomic signatures are also heritable which will allow the use of genetics to study causality of the metabolomic signatures.

Analysis of the metabolomics of individuals with overt and subclinical thyroid disease would be particularly instructive. The key test then will then be to see whether a metabolomics signature of thyroid stress is better correlated with adverse outcomes. In turn having better tissue markers may enable us to have greater insight into the patient’s true thyroid status and lead to better prescribing practice. This may potentially allow better targeting of individuals who might benefit from treatment particularly in those with borderline TSH levels for hypothyroidism. A metabolomic signature of hypothyroidism may be more indicative of intracellular thyroid dysfunction than TSH and may provide clearer data in epidemiological studies as potentially this approach may be more resistant to confounding. Furthermore, this approach may be able to distinguish between higher TSH levels that are a response to low thyroid hormone status rather than common genetic variants which do not appear to have an appreciable effect on FT4 levels. This discrepancy

might be a potential explanation for the differing trial outcomes in subclinical thyroid disease.

It will also be informative to assess whether there is a different metabolomic signature in hypothyroid patients on levothyroxine vs those taking combination T3 and T4 therapy or those on other preparations such as Armour. It is well established that individuals on levothyroxine have a relatively high FT4 level but a relatively low FT3 despite a normal TSH [186]. Assessment of the effect of the different thyroid hormone replacements on metabolomic signature would be a clearer way of demonstrating whether there is any role for alternatives to the standard thyroid hormone replacement of levothyroxine. The role could be extended to iodine deficiency which still occurs in the UK [294] maternal iodine deficiency in pregnancy is associated with reduced offspring IQ [203] due to its effects on the maternal and foetal thyroid. Iodine deficiency is measured by urinary iodine, but this is an imperfect measure heavily influenced by recent diet and is not a good guide of long term iodine status [7]. Metabolomics may allow a better understanding of iodine deficiency and its diagnosis.

8.1 CONCLUSION

As has been highlighted in the discussion of each research chapter in the thesis we have observed that common variation in thyroid hormone has substantial effects on key health outcomes. In this final synthesis of the thesis we see that these effects may be particularly profound during key

periods in life such as pregnancy. What is also readily apparent, is that our current management of hypothyroidism is sub-optimal. Patients are increasingly being started at more modestly elevated TSH levels and are frequently over-treated, the risk benefits of this practice are not fully understood, but our tendency to over-treat young women may result in excess cases of osteoporosis in later life and failure to maintain optimal TSH levels during pregnancy is associated with foetal loss. The management of hypothyroidism could however be extended into screening for sub-optimal thyroid function in pregnancy in an effort to reduce foetal loss.

This thesis has also identified that identifying treatment thresholds from serum thyroid function tests is not straightforward. Rather than defining individuals statistically as having abnormal thyroid function by being outside the 95% reference range an epidemiological approach is more meaningful where abnormal thyroid function is classified as having adverse outcomes that treatment would attenuate or prevent. This may be possible by defining sub-groups based on TPO antibody positivity as in ATA pregnancy guidelines [93] but developing alternative strategies such as the use of metabolomics may yield more valuable insights and the potential for a much greater benefit to risk ratio.

Key advances in thyroid epidemiology have also been made by the Rotterdam group. In particular, their work in pregnancy and also in the elderly has substantially changed our understanding of the field. Work

between our group and the Rotterdam group is becoming increasingly collaborative [295] and will enable with other large meta-analyses of the consequences of variation in thyroid status during pregnancy. Endeavors here could include work on iodine sufficiency and endocrine disruptors in pregnancy [296]. More trial work is needed also to see if very early screening and treating of low thyroid function in pregnancy also improves other obstetric outcomes as well as foetal loss. Data from my CATS and CPRD work have revealed that fetal loss, may be substantially reduced by optimizing thyroid status in early pregnancy. A trial of screening for thyroid dysfunction (subclinical hypothyroidism and isolated hypothyroxinemia) in early pregnancy, taking into account TPO antibody status may reveal substantial benefit in reducing fetal loss. Furthermore additionally measuring beta hCG may help differentiate between a high TSH secondary to thyroid failure versus a high TSH due to low hCG status as seen in a failing placenta. This work may reveal additional benefits of screening and treating for thyroid disease in other areas aside from fetal loss including low birth weight as treatment is likely to be beneficial in mild thyroid failure but unlikely to be beneficial in those with low beta hCG secondary to placental dysfunction. More appropriately powered long-term follow-up trials are needed in subclinical hypothyroidism especially in adults under the age of 70. The work in this thesis has identified that such endeavors are clinically relevant, theoretically possible and urgently needed.

Chapter 9 References

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Appendix 1 Details of socio-economic and early life scores used in the analysis in Chapter 4.

ALSPAC Family Adversity Index

A measure of hardship during pregnancy and early life

Sum of (1 point given for each applicable item):

Demographics

- Mother younger than 20 years at first pregnancy

Housing

- Housing inadequacy (crowding and periods of homelessness)
- Basic living conditions (no availability of hot water, no indoor toilet, bath or shower, or no kitchen)
- Major defects in housing or infestation

Education

- No educational qualifications (mother or father)

Financial status

- Financial difficulties

Relationship with partner

- Single status
- Low affection and aggression
- Physical/emotional cruelty
- No social support
- Family
- Family size >4 children
- Caregiving problems (on social services risk register, child in care/not with natural mother)

Social network

- No emotional support
- No practical/financial support

Maternal emotional status

- Depression, anxiety or suicide attempts
- Substance abuse

Drugs or alcohol use

- Crime

- In trouble with police
- Actual convictions

- **Mother's parenting score**

Assessed at six months postpartum

Variable derived from responses as to how often mother:

- Plays with child
- Sings to child
- Shows child pictures in books
- Plays with toys
- Cuddles child
- Physically plays with child
- Takes child for walks

Home observation for measurement of environment (HOME) score

Assessed at six months postpartum and a measure of the emotional and cognitive environment

Variable derived from responses to:

- Child has cuddly toys
- Child has push/pull toys
- Child has co-ordination toys
- Number of books child has of their own
- Mother teaches child
- Mother talks to child when working

Appendix 2 Pregnancy Read Codes

Read code	Outcome
13H7.00	Unwanted pregnancy
13H8.00	Illegitimate pregnancy
13Hd.00	Teenage pregnancy
13S..00	Pregnancy benefits
13SZ.00	Pregnancy benefit NOS
1514.11	Due to deliver - EDC
1514.12	Estimated date of delivery
250 PG	DIABETES PREGNANCY
2722.00	O/E - breech presentation
275..00	O/E - fetal movements
2752.00	O/E - fetal movements seen
2753.00	O/E - fetal movements felt
275Z.00	O/E - fetal movements NOS
3004B	PREGNANCY DEPRESSION
3049TA	CANNABIS INGESTION IN PREGNANCY
4453.00	Serum pregnancy test positive
44B2.00	Alpha-feto protein normal
4654.00	Urine pregnancy test positive
584..00	Ultrasound in obstetric diagn.
584..11	Fetal U-S scan
584..12	U-S scan - obstetric, diagn.
584..13	Ultra-sound scan - obstetric
5841.00	U-S obstetric scan requested
5842.00	U-S obstetric scan normal
5843.00	U-S obstetric scan abnormal
5844.00	U-S scan -placental localisatn
5844.11	Placenta U-S scan
5845.00	U-S scan - fetal cephalometry
5846.00	U-S scan - fetal maturity
5847.00	U-S scan - fetal abnormality
5848.00	U-S scan - multiple fetus
5849.00	U-S scan - fetal presentation
584B.00	Viability US scan
584C.00	Antenatal ultrasound result received
584D.00	Antenatal ultrasound confirms intra-uterine pregnancy
584G.00	Nuchal scan
584Z.00	U-S obstetric diagn. scan NOS
615C.00	IUD failure - pregnant
615C.11	Pregnant, IUD failure
6166.00	Pregnant, diaphragm failure
6174.00	Pregnant, sheath failure
62...00	Patient pregnant
62...11	Antenatal care

62...12	Maternity care
62...13	Pregnancy care
621..00	Patient currently pregnant
621..11	Pregnancy confirmed
6211.00	Pregnant - urine test confirms
6212.00	Pregnant - blood test confirms
6213.00	Pregnant - V.E. confirms
6215.00	Pregnant - on abdom. palpation
6216.00	Pregnant - planned
6217.00	Pregnant - unplanned - wanted
6218.00	Pregnant -unplanned-not wanted
621A.00	Pregnancy unplanned ? wanted
621B.00	Pregnant - ? planned
621C.00	Unplanned pregnancy
621Z.00	Patient pregnant NOS
622..00	Antenatal care: gravida No.
6221.00	Antenatal care: primigravida
6222.00	Antenatal care: 2nd pregnancy
6223.00	Antenatal care: 3rd pregnancy
6224.00	Antenatal care: multip
622Z.00	Antenatal care: gravida NOS
623..00	A/N care: obstetric risk
6231.00	A/N care: uncertain dates
6232.00	A/N care: recurrent aborter
6233.00	A/N care: grand multip
6234.00	A/N care: H/O stillbirth
6235.00	A/N care: H/O perinatal death
6236.00	A/N care: poor obstetr history
6237.00	A/N care: H/O trophoblast.dis.
623Z.00	A/N care: obstetric risk NOS
624..00	A/N care: precious pregnancy
6241.00	A/N care: elderly primip.
6242.00	A/N care: H/O infertility
624Z.00	A/N care: precious preg. NOS
625..00	A/N care: social risk
6251.00	A/N care: poor home conditions
6252.00	A/N care: poor A/N attender
6253.00	A/N care: late booker
6254.00	A/N care: H/O child abuse
625Z.00	A/N care: social risk NOS
626..00	A/N care: medical risk
627..00	A/N care: gynae. risk
628..00	A/N care: risk NOS
6281.00	A/N care: under 5ft tall
6282.00	A/N care:10yrs+since last preg
6283.00	A/N care: primip. < 17 years
6284.00	A/N care: primip. > 30 years
6285.00	A/N care: multip. > 35 years
628Z.00	A/N risk NOS
629..00	No ante-natal care

6291.00	Ante-natal care: not offered
6292.00	Ante-natal care: not wanted
6293.00	Ante-natal care: not attended
6294.00	No A/N care: not known preg.
629Z.00	No ante-natal care NOS
62A..00	A/N care provider
62A1.00	A/N care from G.P.
62A2.00	A/N care from consultant
62A3.00	A/N - shared care
62A4.00	A/N care midwifery led
62AZ.00	A/N care provider NOS
62B..00	Delivery booking place
62B1.00	Delivery: no place booked
62B2.00	Home delivery booked
62B3.00	G.P. unit delivery booking
62B4.00	Consultant unit booking
62B5.00	Private home delivery booking
62B6.00	Delivery booking place changed
62B8.00	Midwife unit delivery booking
62BZ.00	Delivery booking - place NOS
62C..00	Deliv.booking - length of stay
62C1.00	Short stay delivery booking
62C2.00	Full stay delivery booking
62CZ.00	Delivery booking - stay NOS
62F..00	Antenatal amniocentesis
62F1.00	A/N amniocentesis -not offered
62F2.00	A/N amniocentesis - offered
62F3.00	A/N amniocentesis - not wanted
62F4.00	A/N amniocentesis wanted
62F5.00	A/N amniocentesis - awaited
62F6.00	A/N amniocentesis - normal
62F7.00	A/N amniocentesis - abnormal
62F8.00	A/N amnio. for ? chrom.abnorm.
62F9.00	A/N amnio. for ? neural tube
62FZ.00	Antenatal amniocentesis NOS
62G..00	Antenatal ultrasound scan
62G1.00	A/N U/S scan not offered
62G2.00	A/N U/S scan offered
62G3.00	A/N U/S scan not wanted
62G4.00	A/N U/S scan wanted
62G5.00	A/N U/S scan awaited
62G6.00	A/N U/S scan normal += dates
62G7.00	A/N U/S scan normal +? dates
62G8.00	A/N U/S scan abnormal
62G9.00	A/N U/S scan for ? abnormality
62GA.00	A/N U/S scan for slow growth
62GB.00	Antenatal ultrasounds scan at 4-8 weeks
62GC.00	Antenatal ultrasound scan at 9-16 weeks
62GD.00	Antenatal ultrasound scan at 17-22 weeks
62GE.00	Antenatal ultrasound scan at 22-40 weeks

62GZ.00	Antenatal ultrasound scan NOS
62H..00	A/N Rh antibody screen
62H1.00	A/N Rh screen not offered
62H2.00	A/N Rh screen offered
62H3.00	Rh screen - 1st preg. sample
62H4.00	Rh screen - 2nd preg. sample
62H5.00	Rh screen - 3rd preg. sample
62HZ.00	A/N Rh antibody screen NOS
62I..00	Alpha-feto protein blood test
62I..11	AFP test - antenatal
62I..12	Alpha-feto protein test - A/N
62I1.00	AFP blood test offered
62I2.00	AFP blood test not offered
62I3.00	AFP blood test wanted
62I4.00	AFP blood test not wanted
62I5.00	AFP - blood sent
62IZ.00	AFP blood test NOS
62K..00	Antenatal syphilis screen
62K1.00	A/N syphilis screen not done
62K2.00	A/N syphilis screen-blood sent
62KZ.00	Antenatal syphilis screen NOS
62L..00	Antenatal blood group screen
62L1.00	A/N blood gp screen not done
62L2.00	A/N blood group screen done
62LZ.00	A/N blood group screen NOS
62M..00	Antenatal sickle cell screen
62M1.00	A/N sickle screen not done
62M2.00	A/N sickle cell screen done
62MZ.00	A/N sickle cell screen NOS
62N..00	Antenatal examinations
62N1.00	A/N booking examination
62N2.00	A/N 12 weeks examination
62N3.00	A/N 16 week examination
62N4.00	A/N 20 week examination
62N5.00	A/N 24 week examination
62N6.00	A/N 28 week examination
62N7.00	A/N 30 week examination
62N8.00	A/N 32 week examination
62N9.00	A/N 34 week examination
62NA.00	A/N 35 week examination
62NB.00	A/N 36 week examination
62NC.00	A/N 37 week examination
62ND.00	A/N 38 week examination
62NE.00	A/N 39 week examination
62NF.00	A/N 40 week examination
62NG.00	A/N 41 week examination
62NH.00	A/N 42 week examination
62NZ.00	Antenatal examination NOS
62O..00	Misc. antenatal data
62O..11	Fetal maturity - A/N

62O..12	Static weight gain pregnancy
62O1.00	Fetal movements felt
62O1.11	Quickening
62O2.00	Fetal movements seen
62O3.00	Fetal maturity: dates = size
62O4.00	Fetal maturity: dates not=size
62O6.00	Vaginal 'show'
62O6.11	Vaginal 'show' - A/N
62O7.00	Pregnancy prolonged - 41 weeks
62O8.00	Pregnancy prolonged - 42 weeks
62OZ.00	Misc. antenatal data NOS
62U..00	Downs screen - blood test
62U..11	Barts test
62U..12	Triple test
62U..13	Double test
62U0.00	Triple test offered
62U1.00	Double test offered
62U2.00	Triple test not offered
62U3.00	Double test not offered
62U4.00	Triple test wanted
62U5.00	Double test wanted
62U6.00	Triple test not wanted
62U7.00	Double test not wanted
62U8.00	Downs screening - blood sent
62U9.00	Downs screen blood test normal
62UA.00	Downs screen blood test abnormal
62Uz.00	Downs screening blood test NOS
62V..00	Delivery place planned
62V0.00	Home delivery planned
62W..00	Antenatal blood tests
62X..00	Length of gestation
62X0.00	Gestation <24 weeks
62X1.00	Gestation = 24 weeks
62X2.00	Gestation >24 weeks
62X3.00	Full term gestation - 40 weeks
62Y..00	Routine antenatal care
62Z..00	Maternal care NOS
62a..00	Pregnancy review
62b..00	Antenatal HIV screening
62c..00	Antenatal screening
62u6.00	Triple test not wanted
630	PREGNANCY GENITAL INFECTION
630 B	VAGINITIS PREGNANCY
6320	PREGNANCY PLACENTA PRAEVIA
6320A	PRAEVIA PLACENTA
6320C	PLACENTA PRAEVIA CENTRAL
6320D	PLACENTA PRAEVIA LATERAL
6320E	PLACENTA PRAEVIA MARGINAL
6320F	PLACENTA PRAEVIA PARTIAL
6321BR	PLACENTA ABRUPTIO

6323	THREATENED MISCARRAGE
6329A	PREGNANCY HAEMORRHAGE
6329AB	PREGNANCY BLEEDING
6330	PREGNANCY MACROCYTIC ANAEMIA
6330B	PREGNANCY ANAEMIA MEGALOBLASTIC
6330H	HYPERCHROMIC ANAEMIA PREGNANCY
6331	PREGNANCY IRON-DEFICIENCY ANAEMIA
6331H	PREGNANCY ANAEMIA HYPOCHROMIC
6339	PREGNANCY ANAEMIA
6340	PREGNANCY MALPOSITION FOETUS
6349	PREGNANCY COMPLICATION
6349AA	CONCEALED PREGNANCY
6349AB	PREGNANCY PELVIS BONY ABNORMAL
6349AD	INTRAUTERINE DEATH
6349AP	PREGNANCY ABNORMAL
6349B	TWIN PREGNANCY
6349BM	PREGNANCY MULTIPLE
6349BT	TRIPLET PREGNANCY
6349BV	PREGNANCY BICORNATE UTERUS
6349D	PREGNANCY DISPROPORTION
6349EP	PRIMIGRAVIDA ELDERLY
6349F	VARICOSE VEINS PREGNANCY
6349LF	LABOUR FALSE
6349LG	BRAXTON HICKS CONTRACTIONS
6349LH	FALSE UTERINE CONTRACTIONS
6349LI	HICK'S CONTRACTIONS
6349LP	POSSIBLE LABOUR
6349NF	PREGNANCY INFECTION DURING
6349P	PREGNANCY PHLEBITIS
6349PP	PREGNANCY PHLEBOTHROMBOSIS
6349PT	THROMBOSIS PREGNANCY
6349PV	VARIX COMPLICATING PREGNANCY
6349PW	PREGNANCY MILK LEG
6349SD	SMALL FOR DATES (FOETUS)
6349SR	INTRAUTERINE GROWTH RETARDATION
6349TP	PROLAPSED UTERUS PREGNANCY
6349WE	PREGNANCY WEIGHT GAIN EXCESSIVE
6349WT	STATIC WEIGHT GAIN PREGNANCY
6350CG	PYELOCYSTITIS PREGNANCY
6350G	PYELITIS PREGNANCY
6359A	PREGNANCY CYSTITIS
6359G	URINARY INFECTION PREGNANCY
636 GA	PREGNANCY ALBUMINURIA
636 GM	SYNDROME NEPHROTIC PREGNANCY
636 GN	PREGNANCY NEPHRITIS
6361PG	PREGNANCY GLYCOSURIA
6370	PREGNANCY PRE-ECLAMPSIA
6370A	TOXAEMIA PRE-ECLAMPTIC
6370H	PREGNANCY HYPERTENSION
6370HE	PREGNANCY BP RAISED AT END OF

6371	PREGNANCY ECLAMPSIA
6379	TOXAEMIA PREGNANCY
6389C	PREGNANCY HYPEREMESIS
6389CD	PREGNANCY NAUSEA & VOMITING
6389CK	SICKNESS PREGNANCY
6389CM	PREGNANCY MORNING SICKNESS
6389CP	VOMITING PERNICIOUS PREGNANCY
6389CV	VOMITING PREGNANCY
6389D	PREGNANCY NAUSEA
63C5.00	Maternal tobacco abuse
63C6.00	Maternal drug abuse
6409TR	TERMINATION OF PREGNANCY REQUESTED
651 G	PREMATURE SEPARATION PLACENTA
655 C	DISPROPORTION CEPHALOPELVIC
656 BP	PRESENTATION BREECH (MOTHER)
6600C	UTERINE PERFORATION OBSTETRICAL
661 K	LABOUR PREMATURE WITH COMPLICATIONS
66AX.00	Diabetes: shared care in pregnancy - diabetol and obstet
6776.00	Preg. termination counselling
679E.00	Antenatal education
67A2.00	Diet in pregnancy advice
67A3.00	Pregnancy smoking advice
67A4.00	Pregnancy exercise advice
67A5.00	Pregnancy alcohol advice
67A6.00	Drugs in pregnancy advice
67A7.00	Pregnancy dental advice
67A7.11	Care of teeth advice -in preg.
67A8.00	Maternity grant advice
67AA.00	Maternity milk/vits advice
67AB.00	Preg. prescription exempt adv.
67B..00	Ante-natal relaxation classes
6981BP	PRURITUS OF PREGNANCY
7615	PREGNANCY ACCIDENT AFFECTING BABY
7763	FOETAL DISTRESS
7763A	ASPHYXIA ANTENATAL
7763DM	DECREASED FOETAL MOVEMENTS
7763FM	FOETAL MOVEMENTS NOT FELT
7763MD	FOETAL MOVEMENTS DECREASED
7789C	ACCIDENT INTRAUTERINE FOETUS/NEWBORN
7E06000	Open removal of products of conception from uterus NEC
7F...00	Obstetric operations
7F...12	Pregnancy operations
7F0..00	Fetus and gravid uterus operations
7F0..11	Fetus operations
7F0..12	Fetus & gravid uterus ops
7F00.00	Therapeutic fetoscopic operations on fetus
7F00.11	Therapeutic endoscopic operations on fetus
7F00.12	Therapeutic foetoscopic operations on fetus

7F00000	Fetoscopic blood transfusion of fetus
7F00y00	Other specified therapeutic fetoscopic operation
7F00z00	Therapeutic fetoscopic operation NOS
7F01.00	Diagnostic endoscopic examination of fetus using fetoscope
7F01.11	Diagnostic endoscopic examination of foetus using fetoscope
7F01000	Fetoscopic examination of fetus and biopsy of fetus
7F01100	Fetoscopic examination of fetus and sampling of fetal blood
7F01111	Foetoscopic examination foetus and sampling of foetal blood
7F01y00	Diagnostic endoscopic examination fetus using fetoscope OS
7F01z00	Diagnostic endoscopic examination fetus using fetoscope NOS
7F01z11	Diagnost endoscopic examination foetus using foetoscope NOS
7F01z12	Fetoscopy NEC
7F03.00	Therapeutic percutaneous operations on fetus
7F03000	Percutaneous insertion of fetal vesicoamniotic shunt
7F03100	Percutaneous insertion of fetal pleuroamniotic shunt
7F03200	Percutaneous blood transfusion of fetus
7F03y00	Other specified therapeutic percutaneous operation on fetus
7F03z00	Therapeutic percutaneous operation on fetus NOS
7F04.00	Diagnostic percutaneous examination of fetus
7F04.11	Diagnostic percutaneous examination of placenta
7F04000	Percutaneous biopsy of fetus
7F04100	Percutaneous sampling of fetal blood
7F04111	Percutaneous sampling of foetal blood
7F04200	Percutaneous sampling of chorionic villus
7F04y00	Other specified diagnostic percutaneous examination of fetus
7F04z00	Diagnostic percutaneous examination of fetus NOS
7F05.00	Other operations on amniotic cavity
7F05000	Drainage of amniotic cavity
7F05100	Diagnostic amniocentesis
7F05111	Amniocentesis NEC
7F05200	Amnioscopy
7F05300	Sampling of chorionic villus NEC
7F05y00	Other specified other operation on amniotic cavity
7F05z00	Other operation on amniotic cavity NOS
7F06.00	Operations on gravid uterus
7F06000	Cerclage of cervix of gravid uterus
7F06011	McDonald cerclage of cervix
7F06012	Shirodkar suture in pregnancy
7F06100	Removal of cerclage from cervix of gravid uterus

7F06111	Removal of Shirodkar suture
7F06200	Repositioning of retroverted gravid uterus
7F06300	External version of breech
7F06y00	Other specified operation on gravid uterus
7F06z00	Operation on gravid uterus NOS
7F0y.00	Other specified operations on fetus or gravid uterus
7F0z.00	Fetus and gravid uterus operations NOS
7F1A300	Drainage of hydrocephalus of fetus to facilitate delivery
7F1Bz00	Other operation to facilitate delivery NOS
7F2..00	Other obstetric operations
7F24.00	Other obstetric operations
7F24y00	Other specified other obstetric operation
7F24z00	Other obstetric operation NOS
7F25.00	Obstetric monitoring
7F25.11	Fetal monitoring
7F25.12	Foetal monitoring
7F25000	Fetal heart monitoring NEC
7F25z00	Obstetric monitoring NOS
7F2y.00	Other specified obstetric operations
7F2z.00	Other obstetric operations NOS
7Fy..00	Other specified obstetric operations
8B68.00	Pregnancy prophylactic therapy
8B7..11	Pregnancy vitamin/iron prophyl
8B74.00	Iron supplement in pregnancy
8B75.00	Vitamin supplement - pregnancy
8E96.00	Ante-natal exercises
8H7W.00	Refer to TOP counselling
8HHV.00	Referral for termination of pregnancy
8HHf.00	Refer to early pregnancy unit
8HT9.00	Referral to antenatal clinic
8HV6.00	Private referral to obstetrician
8M6..00	Requests pregnancy termination
95...00	Maternity services admin.
951..00	FP24 maternity claim status
9Ea..00	Reason for termination of pregnancy
9Ea0.00	Risk life pregnant woman greater than if pregnancy terminatd
9Ea1.00	To prevent grave permnt inj physic/mental health preg woman
9Ea2.00	Less 24 wk involv risk injury physic/mentl health preg woman
9Ea3.00	Lss 24 wk inv risk inj phys/men hlth ext child preg wom fmly
9Ea4.00	Unborn child at risk physi/ment abnormal serious handicap
9N1N.00	Seen in antenatal clinic
K7071AB	SUTURE SHIRODKAR
K744	PREGNANT HYSTERECTOMY
K746	AMNIOCENTESIS

K7461CV	CHORIONIC VILLOUS SAMPLING
K748 AP	ANTENATAL OPERATION
K752 AA	REPOSITIONING FOETUS
K759	ANTEPARTUM OPERATION
K965 ME	VERSION EXTERNAL
L 115H	ALPHA-FETO PROTEIN HIGH
L 115L	ALPHA-FETO PROTEIN LOW
L 115N	ALPHA-FETO PROTEIN NORMAL
L 134C	AZ TEST POSITIVE
L 134DA	HUMAN PLACENTAL LACTOGEN LEVEL ABNORMAL
L 134DN	HUMAN PLACENTAL LACTOGEN LEVEL NORMAL
L 134FA	PLACENTAL FUNCTION TEST
L 134FB	PLACENTAL FUNCTION TEST NORMAL
L 134FC	PLACENTAL FUNCTION TEST ABNORMAL
L 134P	PREGNANCY TEST POSITIVE
L0010BE	SEEN IN ANTENATAL CLINIC
L0010FE	REFERRED TO ANTENATAL CLINIC
L01z.00	Other abnormal product of conception NOS
L031100	Gravid fallopian tube rupture
L03y000	Cervical pregnancy
L03y100	Cornual pregnancy
L03y200	Membranous pregnancy
L03y300	Combined or heterotopic pregnancy
L03y400	Mural pregnancy
L03y500	Intraligamentous pregnancy
L03y600	Mesenteric pregnancy
L03y700	Angular pregnancy
L03y800	Mesometric pregnancy
L08..00	Failed attempted abortion
L080.00	Failed attempted abortion + genital tract/pelvic infection
L081.00	Failed attempted abortion + delayed or excessive haemorrhage
L082.00	Failed attempted abortion + damage to pelvic organs/tissues
L083.00	Failed attempted abortion with renal failure
L084.00	Failed attempted abortion with metabolic disorder
L085.00	Failed attempted abortion with shock
L086.00	Failed attempted abortion with embolism
L08w.00	Failed attempted abortion with other specified complication
L08x.00	Failed attempted abortion with complication NOS
L08y.00	Failed attempted abortion with no mention of complication
L08z.00	Failed attempted abortion NOS
L0A..00	Failed attempted abortion
L0A1.00	Failed medical abortion complic by genital tract/pelvic infn
L0A2.00	Failed medical abortion comp by delayed/excessive haem'ge

L0A3.00	Failed medical abortion, complicated by embolism
L0A4.00	Failed medical abortion, without complication
L1...00	Pregnancy complications
L10..00	Haemorrhage in early pregnancy
L100.00	Threatened abortion
L100000	Threatened abortion unspecified
L100200	Threatened abortion - not delivered
L100z00	Threatened abortion NOS
L10y.00	Other haemorrhage in early pregnancy
L10y.11	Bleeding in early pregnancy
L10y000	Other haemorrhage in early pregnancy unspecified
L10y200	Other haemorrhage in early pregnancy - not delivered
L10yz00	Other haemorrhage in early pregnancy NOS
L10z.00	Early pregnancy haemorrhage NOS
L10z000	Early pregnancy haemorrhage NOS unspecified
L10z200	Early pregnancy haemorrhage NOS - not delivered
L10zz00	Early pregnancy haemorrhage NOS
L11..00	Antepartum haemorrhage, abruptio placentae, placenta praevia
L11..11	Antepartum haemorrhage
L11..12	Antepartum bleeding
L110.00	Placenta praevia without haemorrhage
L110000	Placenta praevia without haemorrhage unspecified
L110200	Placenta praevia without haemorrhage - not delivered
L110z00	Placenta praevia without haemorrhage NOS
L111.00	Placenta praevia with haemorrhage
L111000	Placenta praevia with haemorrhage unspecified
L111200	Placenta praevia with haemorrhage - not delivered
L111z00	Placenta praevia with haemorrhage NOS
L112.00	Placental abruption
L112.11	Ablatio placentae
L112.12	Couvelaire uterus
L112000	Placental abruption unspecified
L112200	Placental abruption - not delivered
L112300	Premature separation of placenta with coagulation defect
L112z00	Placental abruption NOS
L113.00	Antepartum haemorrhage with coagulation defect
L113.11	Antepartum haemorrhage with afibrinogenaemia
L113.12	Antepartum haemorrhage with hyperfibrinolysis
L113.13	Antepartum haemorrhage with hypofibrinogenaemia
L113000	Antepartum haemorrhage with coagulation defect unspecified
L113200	Antepartum haemorrhage with coagulation defect - not deliv
L113z00	Antepartum haemorrhage with coagulation defect NOS

L114.00	Antepartum haemorrhage with trauma
L114000	Antepartum haemorrhage with trauma unspecified
L114200	Antepartum haemorrhage with trauma - not delivered
L114z00	Antepartum haemorrhage with trauma NOS
L115.00	Antepartum haemorrhage with uterine leiomyoma
L115.11	Antepartum haemorrhage with fibroid
L115.12	Antepartum haemorrhage with uterine fibroid
L115000	Antepartum haemorrhage with uterine leiomyoma unspecified
L115200	Antepartum haemorrhage with uterine leiomyoma - not deliv
L115z00	Antepartum haemorrhage with uterine leiomyoma NOS
L116.00	Placenta praevia
L11y.00	Other antepartum haemorrhage
L11y000	Other antepartum haemorrhage unspecified
L11y200	Other antepartum haemorrhage - not delivered
L11yz00	Other antepartum haemorrhage NOS
L11z.00	Antepartum haemorrhage NOS
L11z000	Antepartum haemorrhage NOS, unspecified
L11z200	Antepartum haemorrhage NOS - not deliv
L11zz00	Antepartum haemorrhage NOS
L120300	Benign essential hypertension in preg/childb/puerp-not deliv
L121300	Renal hypertension in preg/childbirth/puerp - not delivered
L122300	Other pre-exist hypertension in preg/childb/puerp-not deliv
L123.00	Transient hypertension of pregnancy
L123000	Transient hypertension of pregnancy unspecified
L123300	Transient hypertension of pregnancy - not delivered
L123500	Gestational hypertension
L123600	Transient hypertension of pregnancy
L123z00	Transient hypertension of pregnancy NOS
L124.00	Mild or unspecified pre-eclampsia
L124.11	Mild pre-eclampsia
L124.12	Toxaemia NOS
L124000	Mild or unspecified pre-eclampsia unspecified
L124300	Mild or unspecified pre-eclampsia - not delivered
L124500	Mild pre-eclampsia
L124600	Pre-eclampsia, unspecified
L124z00	Mild or unspecified pre-eclampsia NOS
L125.00	Severe pre-eclampsia
L125000	Severe pre-eclampsia unspecified
L125300	Severe pre-eclampsia - not delivered
L125z00	Severe pre-eclampsia NOS
L126.00	Eclampsia
L126000	Eclampsia unspecified
L126300	Eclampsia - not delivered

L126500	Eclampsia in pregnancy
L126z00	Eclampsia NOS
L127.00	Pre-eclampsia or eclampsia with pre-existing hypertension
L127000	Pre-eclampsia or eclampsia with hypertension unspecified
L127300	Pre-eclampsia or eclampsia with hypertension - not delivered
L127z00	Pre-eclampsia or eclampsia + pre-existing hypertension NOS
L129.00	Moderate pre-eclampsia
L12A.00	HELLP - Syndrome haemolysis, elev liver enzyme low platelets
L12B.00	Proteinuric hypertension of pregnancy
L13..00	Excessive pregnancy vomiting
L13..11	Hyperemesis gravidarum
L13..12	Hyperemesis of pregnancy
L130.00	Mild hyperemesis gravidarum
L130.11	Morning sickness
L130000	Mild hyperemesis unspecified
L130200	Mild hyperemesis-not delivered
L130z00	Mild hyperemesis gravidarum NOS
L131.00	Hyperemesis gravidarum with metabolic disturbance
L131000	Hyperemesis gravidarum with metabolic disturbance unsp
L131200	Hyperemesis gravidarum with metabolic disturbance - not del
L131z00	Hyperemesis gravidarum with metabolic disturbance NOS
L132.00	Late vomiting of pregnancy
L132000	Late pregnancy vomiting unspecified
L132200	Late pregnancy vomiting - not delivered
L132z00	Late pregnancy vomiting NOS
L13y.00	Other pregnancy vomiting
L13y000	Other pregnancy vomiting unspecified
L13y200	Other pregnancy vomiting - not delivered
L13yz00	Other pregnancy vomiting NOS
L13z.00	Unspecified pregnancy vomiting
L13z000	Unspecified pregnancy vomiting unspecified
L13z200	Unspecified pregnancy vomiting - not delivered
L13zz00	Unspecified pregnancy vomiting NOS
L14..00	Early or threatened labour
L140.00	Threatened premature labour
L140.11	False labour
L140000	Threatened premature labour unspecified
L140100	Threatened premature labour - not delivered
L140200	False labour at or after 37 completed weeks of gestation
L140z00	Threatened premature labour NOS

L141.00	Other threatened labour
L141000	Other threatened labour unspecified
L141100	Other threatened labour - not delivered
L141z00	Other threatened labour NOS
L14z.00	Early or threatened labour NOS
L15..00	Prolonged or post-term pregnancy
L15..11	Post-term pregnancy
L150.00	Post-term pregnancy
L150000	Post-term pregnancy unspecified
L150200	Post-term pregnancy - not delivered
L150z00	Post-term pregnancy NOS
L15z.00	Prolonged pregnancy NOS
L16..00	Other pregnancy complication NEC
L160.00	Papyraceous fetus
L160000	Papyraceous fetus unspecified
L160200	Papyraceous fetus - not delivered
L160z00	Papyraceous fetus NOS
L161.00	Oedema or excessive weight gain in pregnancy no hypertension
L161.11	Excessive weight gain in pregnancy
L161.12	Maternal obesity syndrome
L161.13	Gestational oedema
L161000	Oedema or excessive weight gain in pregnancy, unspecified
L161300	Oedema or excessive weight gain in pregnancy - not delivered
L161z00	Oedema or excessive weight gain in pregnancy NOS
L162.00	Unspecified renal disease in pregnancy
L162.11	Albuminuria in pregnancy without hypertension
L162.12	Nephropathy NOS in pregnancy without hypertension
L162.13	Uraemia in pregnancy without hypertension
L162000	Unspecified renal disease in pregnancy unspecified
L162300	Unspecified renal disease in pregnancy - not delivered
L162z00	Unspecified renal disease in pregnancy NOS
L163200	Habitual aborter - not delivered
L163300	Pregnancy care of habitual aborter
L164.00	Peripheral neuritis in pregnancy
L164000	Peripheral neuritis in pregnancy unspecified
L164300	Peripheral neuritis in pregnancy - not delivered
L164z00	Peripheral neuritis in pregnancy NOS
L165.00	Asymptomatic bacteriuria in pregnancy
L165000	Asymptomatic bacteriuria in pregnancy unspecified
L165300	Asymptomatic bacteriuria in pregnancy - not delivered
L165z00	Asymptomatic bacteriuria in pregnancy NOS
L166.00	Genitourinary tract infections in pregnancy
L166.11	Cystitis of pregnancy

L166000	Genitourinary tract infection in pregnancy unspecified
L166300	Genitourinary tract infection in pregnancy - not delivered
L166500	Infections of kidney in pregnancy
L166700	Infections of the genital tract in pregnancy
L166800	Urinary tract infection complicating pregnancy
L166z00	Genitourinary tract infection in pregnancy NOS
L166z11	UTI - urinary tract infection in pregnancy
L167.00	Liver disorder in pregnancy
L167000	Liver disorder in pregnancy unspecified
L167200	Liver disorder in pregnancy - not delivered
L167z00	Liver disorder in pregnancy NOS
L168.00	Fatigue during pregnancy
L168000	Fatigue during pregnancy unspecified
L168300	Fatigue during pregnancy - not delivered
L168z00	Fatigue during pregnancy NOS
L169300	Herpes gestationis - not delivered
L16A.00	Glycosuria during pregnancy
L16A000	Glycosuria during pregnancy unspecified
L16A300	Glycosuria during pregnancy - not delivered
L16Az00	Glycosuria during pregnancy NOS
L16B.00	Braxton-Hicks contractions
L16C.00	Pregnancy induced oedema+proteinuria without hypertension
L16C000	Gestational proteinuria
L16C100	Gestational oedema with proteinuria
L16D.00	Excessive weight gain in pregnancy
L16E.00	Pregnancy pruritus
L16y.00	Other pregnancy complications
L16y000	Other pregnancy complication unspecified
L16y300	Other pregnancy complication - not delivered
L16y500	Abdominal pain in pregnancy
L16yz00	Other pregnancy complication NOS
L16z.00	Pregnancy complication NOS
L170300	Maternal syphilis during pregnancy - baby not yet delivered
L171300	Maternal gonorrhoea in pregnancy - baby not yet delivered
L172300	Other maternal venereal dis. in pregnancy-baby not delivered
L173300	Maternal tuberculosis in pregnancy - baby not yet delivered
L174300	Maternal malaria during pregnancy - baby not yet delivered
L175.11	Rubella contact in pregnancy
L175300	Maternal rubella during pregnancy - baby not yet delivered
L176300	Other maternal viral dis.in pregnancy-baby not yet delivered

L177.00	Infections of bladder in pregnancy
L178.00	Infections of urethra in pregnancy
L17y300	Other mat infective/parasit dis in pregnancy - not delivered
L17z300	Mat infect/parasitic dis NOS in pregnancy-baby not delivered
L180300	Diabetes mellitus during pregnancy - baby not yet delivered
L180800	Diabetes mellitus arising in pregnancy
L180811	Gestational diabetes mellitus
L180900	Gestational diabetes mellitus
L181300	Thyroid dysfunction in pregnancy - baby not yet delivered
L182300	Anaemia during pregnancy - baby not yet delivered
L182500	Iron deficiency anaemia of pregnancy
L183.11	Pregnancy and drug dependence
L183300	Drug dependence during pregnancy - baby not yet delivered
L184300	Mental disorder during pregnancy - baby not yet delivered
L185.11	Congenital heart disease in pregnancy
L185300	Congenital cardiovasc dis in pregnancy - baby not delivered
L186.11	Heart disease during pregnancy
L186300	Other cardiovascular dis in pregnancy - baby not delivered
L187300	Orthopaedic disorder in pregnancy - baby not yet delivered
L188300	Abnormal GTT during pregnancy - baby not yet delivered
L18A000	Cholestasis of pregnancy
L18z300	Medical condition NOS in pregnancy - baby not yet delivered
L19..00	Complications specific to multiple gestation
L191.00	Continuing pregnancy after abortion of one fetus or more
L192.00	Continuing preg after intrauterine death one fetus or more
L2...00	Risk factors in pregnancy
L21..00	Multiple pregnancy
L21..11	Gestation - multiple
L210.00	Twin pregnancy
L210000	Twin pregnancy unspecified
L210200	Twin pregnancy with antenatal problem
L210z00	Twin pregnancy NOS
L211.00	Triplet pregnancy
L211000	Triplet pregnancy unspecified
L211200	Triplet pregnancy with antenatal problem
L211z00	Triplet pregnancy NOS
L212.00	Quadruplet pregnancy

L212000	Quadruplet pregnancy unspecified
L212200	Quadruplet pregnancy with antenatal problem
L212z00	Quadruplet pregnancy NOS
L21y.00	Other multiple pregnancy
L21y000	Other multiple pregnancy unspecified
L21y200	Other multiple pregnancy with antenatal problem
L21yz00	Other multiple pregnancy NOS
L21z.00	Multiple pregnancy NOS
L21z000	Multiple pregnancy NOS, unspecified
L21z200	Multiple pregnancy NOS with antenatal problem
L21zz00	Multiple pregnancy NOS
L22..00	Malposition and malpresentation of fetus
L22..11	Malpresentation of fetus
L220.00	Fetus - unstable lie
L220000	Unstable lie unspecified
L220200	Unstable lie with antenatal problem
L220z00	Unstable lie NOS
L221.00	Cephalic version NOS
L221000	Cephalic version NOS, unspecified
L221200	Cephalic version NOS with antenatal problem
L221z00	Cephalic version NOS
L222.00	Breech presentation
L222000	Breech presentation unspecified
L222200	Breech presentation with antenatal problem
L222z00	Breech presentation NOS
L223.00	Oblique presentation
L223000	Oblique lie unspecified
L223200	Oblique lie with antenatal problem
L223z00	Oblique lie NOS
L224.00	Transverse presentation
L224000	Transverse lie unspecified
L224200	Transverse lie with antenatal problem
L224z00	Transverse lie NOS
L227.00	High head at term
L227000	High head at term unspecified
L227200	High head at term with antenatal problem
L227z00	High head at term NOS
L228.00	Multiple pregnancy with malpresentation
L228000	Multiple pregnancy with malpresentation unspecified
L228200	Multiple pregnancy with malpresentation with antenatal prob
L228z00	Multiple pregnancy with malpresentation NOS
L22y.00	Other fetal malposition and malpresentation
L22y000	Other fetal malposition and malpresentation unspecified
L22y200	Other fetal malposition and malpresentation with a/n prob
L22yz00	Other fetal malposition and malpresentation NOS
L22z.00	Fetal malposition and malpresentation NOS

L22z000	Fetal malposition and malpresentation NOS, unspecified
L22z200	Fetal malposition and malpresentation NOS with a/n problem
L22zz00	Fetal malposition and malpresentation NOS
L23..00	Cephalo-pelvic disproportion
L230.00	Disproportion - major pelvic abnormality
L230000	Disproportion - major pelvic abnormality unspecified
L230200	Disproportion - major pelvic abnormality with antenatal prob
L230z00	Disproportion - major pelvic abnormality NOS
L231.00	Generally contracted pelvis
L231000	Generally contracted pelvis unspecified
L231200	Generally contracted pelvis with antenatal problem
L231z00	Generally contracted pelvis NOS
L232.00	Inlet pelvic contraction
L232000	Inlet pelvic contraction unspecified
L232200	Inlet pelvic contraction with antenatal problem
L232z00	Inlet pelvic contraction NOS
L233.00	Outlet pelvic contraction
L233000	Outlet pelvic contraction unspecified
L233200	Outlet pelvic contraction with antenatal problem
L233z00	Outlet pelvic contraction NOS
L234.00	Mixed feto-pelvic disproportion
L234000	Mixed feto-pelvic disproportion unspecified
L234200	Mixed feto-pelvic disproportion with antenatal problem
L234z00	Mixed feto-pelvic disproportion NOS
L235.00	Large fetus causing disproportion
L235000	Large fetus causing disproportion unspecified
L235200	Large fetus causing disproportion with antenatal problem
L235z00	Large fetus causing disproportion NOS
L236.00	Hydrocephalic disproportion
L236000	Hydrocephalic disproportion unspecified
L236200	Hydrocephalic disproportion with antenatal problem
L236z00	Hydrocephalic disproportion NOS
L237.00	Other fetal abnormality causing disproportion
L237.11	Conjoined twins causing disproportion
L237000	Other fetal abnormality causing disproportion unspecified
L237200	Other fetal abnormality causing disproportion with a/n prob
L237z00	Other fetal abnormality causing disproportion NOS
L23y.00	Other disproportion
L23y000	Other disproportion unspecified
L23y200	Other disproportion with antenatal problem
L23yz00	Other disproportion NOS

L23z.00	Disproportion NOS
L23z000	Disproportion NOS, unspecified
L23z200	Disproportion NOS with antenatal problem
L23zz00	Disproportion NOS
L240000	Congenital abnormality of uterus affecting obstetric care
L240011	Bicornuate uterus affecting obstetric care
L240300	Cong abnorm uterus complicating a/n care, baby not delivered
L240311	Bicornuate uterus complicating a/n care, baby not delivered
L241000	Tumour of uterine body affecting obstetric care
L241011	Uterine fibroid affecting obstetric care
L241300	Tumour of uterine body complicating a/n care, baby not deliv
L241311	Uterine fibroid complicating a/n care, baby not delivered
L243.00	Retroverted incarcerated gravid uterus
L243000	Retroverted incarcerated gravid uterus unspecified
L243300	Retroverted incarcerated gravid uterus with antenatal prob
L243z00	Retroverted incarcerated gravid uterus NOS
L244011	Cystocele affecting obstetric care
L244300	Other uterine/pelvic floor abnormal - baby not yet delivered
L244311	Cystocele complicating antenatal care - baby not delivered
L244312	Rectocele complicating antenatal care - baby not delivered
L245.00	Cervical incompetence
L245.11	Shirodkar suture present
L245000	Cervical incompetence unspecified
L245300	Cervical incompetence with antenatal problem
L245z00	Cervical incompetence NOS
L246000	Other cervical abnormality affecting obstetric care
L246300	Other cervical abn complicating a/n care- baby not delivered
L246311	Polyp of cervix complicating a/n care- baby not delivered
L246312	Stenosis of cervix complicating a/n care- baby not delivered
L247300	Vaginal abnormality complicating a/n care-baby not delivered
L247311	Septate vagina complicating a/n care- baby not yet delivered
L247312	Stenosis of vagina complicating a/n care- baby not delivered
L248012	Rigid perineum affecting obstetric care
L248300	Vulval abn complicating a/n care - baby not yet delivered

L248311	Persistent hymen complicating a/n care - baby not delivered
L248312	Rigid perineum complicating a/n care - baby not delivered
L248z12	Rigid perineum in pregnancy/childbirth/puerperium NOS
L25..00	Known or suspected fetal abnormality
L250.00	Fetus with central nervous system malformation
L250.11	Suspect fetal anencephaly
L250.12	Suspect fetal hydrocephaly
L250.13	Suspect fetal spina bifida
L250000	Fetus with central nervous system malformation unspecified
L250200	Fetus with central nervous system malformation + a/n problem
L250300	Maternal care for suspected CNS malformation in fetus
L250400	Maternal care for CNS malformation in fetus
L250z00	Fetus with central nervous system malformation NOS
L251.00	Fetus with chromosomal abnormality
L251.11	Suspect cystic fibrosis fetus
L251.12	Suspect mongol fetus
L251000	Fetus with chromosomal abnormality unspecified
L251200	Fetus with chromosomal abnormality with antenatal problem
L251300	Maternal care for suspected chromosomal abnormality in fetus
L251400	Maternal care for chromosomal abnormality in fetus
L251z00	Fetus with chromosomal abnormality NOS
L252.00	Fetus with hereditary disease
L252000	Fetus with hereditary disease unspecified
L252200	Fetus with hereditary disease with antenatal problem
L252z00	Fetus with hereditary disease NOS
L253.00	Fetus with viral damage via mother
L253.11	Fetus with suspected rubella damage via mother
L253000	Fetus with viral damage via mother unspecified
L253200	Fetus with viral damage via mother with antenatal problem
L253300	Maternal care for damage to fetus from maternal rubella
L253z00	Fetus with viral damage via mother NOS
L254.00	Fetus with damage due to other maternal disease
L254.11	Suspect fetal damage from maternal alcohol
L254.12	Suspect fetal damage from maternal toxoplasmosis
L254000	Fetus with damage due to other maternal disease unspecified
L254200	Fetus with damage due to other maternal disease + a/n prob

L254z00	Fetus with damage due to other maternal disease NOS
L255.00	Fetus with drug damage
L255000	Fetus with drug damage unspecified
L255200	Fetus with drug damage with antenatal problem
L255300	Maternal care for (suspected) damage to fetus from alcohol
L255z00	Fetus with drug damage NOS
L256.00	Fetus with radiation damage
L256000	Fetus with radiation damage unspecified
L256200	Fetus with radiation damage with antenatal problem
L256z00	Fetus with radiation damage NOS
L257.00	Fetus with damage due to intra-uterine contraceptive device
L257.11	Fetus with damage due to coil
L257.12	Fetus with damage due to intra-uterine contraceptive device
L257000	Fetus with damage due to IUCD unspecified
L257200	Fetus with damage due to IUCD with antenatal problem
L257z00	Fetus with damage due to IUCD NOS
L25y.00	Fetus with other damage NEC
L25y000	Fetus with other damage NEC, unspecified
L25y200	Fetus with other damage NEC with antenatal problem
L25yz00	Fetus with other damage NEC NOS
L25z.00	Fetus with damage NOS
L25z000	Fetus with damage NOS, unspecified
L25z200	Fetus with damage NOS with antenatal problem
L25z300	Maternal care for suspect fetal abnormal and damage, unspec
L25z400	Maternal care for fetal abnormality and damage, unspecified
L25zz00	Fetus with damage NOS
L26..00	Other fetal and placental problems
L260.00	Fetal-maternal haemorrhage
L260000	Fetal-maternal haemorrhage unspecified
L260200	Fetal-maternal haemorrhage with antenatal problem
L260z00	Fetal-maternal haemorrhage NOS
L261200	Rhesus isoimmunisation with antenatal problem
L262200	Other blood-group isoimmunisation with antenatal problem
L263.00	Fetal distress - affecting management
L263.11	Fetal acidosis
L263.12	Fetal bradycardia
L263.13	Fetal tachycardia
L263000	Fetal distress unspecified
L263200	Fetal distress with antenatal problem

L263311	Maternal care for fetal hypoxia
L263700	Maternal care for fetal hypoxia
L263900	Maternal care for fetal tachycardia during pregnancy
L263A00	Maternal care for fetal bradycardia during pregnancy
L263A11	Maternal care for reduced fetal heart rate during pregnancy
L263B00	Maternal care for fetal acidosis during pregnancy
L263z00	Fetal distress NOS
L264.00	Intrauterine death
L264.11	Fetal death in utero
L264000	Intrauterine death unspecified
L264200	Intrauterine death with antenatal problem
L264z00	Intrauterine death NOS
L265.00	Small-for-dates fetus in pregnancy
L265.11	Placental insufficiency
L265200	Small-for-dates with antenatal problem
L265300	Maternal care for poor fetal growth
L265311	Maternal care for intrauterine growth retardation
L266.00	Large-for-dates fetus in pregnancy
L266200	Large-for-dates with antenatal problem
L266300	Suspected macroscopic fetus
L267300	Placental transfusion syndromes
L267500	Other fetal problems
L268.00	Other fetal problems
L268000	Reduced fetal movements
L26y.00	Other feto-placental problems
L26y000	Other feto-placental problems unspecified
L26y200	Other feto-placental problems with antenatal problem
L26yz00	Other feto-placental problems NOS
L26z.00	Feto-placental problems NOS
L26z000	Feto-placental problems NOS, unspecified
L26z200	Feto-placental problems NOS with antenatal problem
L26zz00	Feto-placental problems NOS
L27..00	Polyhydramnios and hydramnios
L27..11	Hydramnios
L270.00	Polyhydramnios
L270000	Polyhydramnios unspecified
L270200	Polyhydramnios with antenatal problem
L270z00	Polyhydramnios NOS
L27z.00	Polyhydramnios NOS
L28..00	Other problems of amniotic cavity and membranes
L280.00	Oligohydramnios
L280000	Oligohydramnios unspecified
L280200	Oligohydramnios with antenatal problem
L280300	Anhydramnios
L280z00	Oligohydramnios NOS

L284.00	Amniotic cavity infection
L284.11	Amnionitis
L284.13	Membranitis
L284.14	Placentitis
L284000	Amniotic cavity infection unspecified
L284200	Amniotic cavity infection with antenatal problem
L284z00	Amniotic cavity infection NOS
L28y.00	Other problems of amniotic cavity and membranes
L28y.11	Amnion nodosum
L28y.12	Amniotic cyst
L28y000	Other problem of amniotic cavity and membranes unspecified
L28y200	Other amniotic/membrane problem with antenatal problem
L28yz00	Other problem of amniotic cavity and membranes NOS
L28z.00	Amniotic cavity and membrane problems NOS
L28z000	Amniotic cavity and membrane problem NOS, unspecified
L28z200	Amniotic cavity and membrane problem NOS with a/n problem
L28zz00	Amniotic cavity and membrane problem NOS
L295.00	Elderly primigravida
L295000	Elderly primigravida unspecified
L295200	Elderly primigravida with antenatal problem
L295z00	Elderly primigravida NOS
L2A..00	Abnormal findings on antenatal screening of mother
L2A0.00	Abnormal haematologic find on antenatal screening of mother
L2A1.00	Abnormal biochemical finding on antenatal screen of mother
L2A2.00	Abnormal cytological finding on antenatal screen of mother
L2A3.00	Abnormal ultrasonic finding on antenatal screening of mother
L2A4.00	Abnormal radiological finding on antenatal screen of mother
L2A5.00	Abnormal chromosomal and genet find/antenat screen of mother
L2AX.00	Abnormal finding on antenatal screening of mother
L2B..00	Low weight gain in pregnancy
L2C..00	Malnutrition in pregnancy
L2D..00	Retained intrauterine contraceptive device in pregnancy
L2y..00	Other specified risk factors in pregnancy
L2z..00	Risk factors in pregnancy NOS
L304.00	Persistent occipitoposterior or occipitoanterior position
L304000	Persistent occipitopost/occipitoant position, unspecified

L3210CF	CONTRACEPTION CAP FAILURE
L3241SF	CONTRACEPTION SHEATH FAILURE
L33..00	Umbilical cord complications
L335.00	Vasa praevia
L335.11	Velamentous insertion of cord
L335000	Vasa praevia unspecified
L335200	Vasa praevia with antenatal problem
L335z00	Vasa praevia NOS
L336.00	Vascular lesions of cord
L336000	Vascular lesions of cord unspecified
L336200	Vascular lesions of cord with antenatal problem
L336z00	Vascular lesions of cord NOS
L33y.00	Other umbilical cord complications
L33y000	Other umbilical cord complications unspecified
L33y200	Other umbilical cord complications with antenatal problem
L33yz00	Other umbilical cord complications NOS
L33z.00	Umbilical cord complications NOS
L33z000	Umbilical cord complications NOS, unspecified
L33z200	Umbilical cord complications NOS with antenatal problem
L33zz00	Umbilical cord complications NOS
L370.11	Placenta accreta without haemorrhage
L385.00	Failed or difficult intubation during pregnancy
L390.00	Maternal distress
L390000	Maternal distress unspecified
L390300	Maternal distress with antenatal problem
L390z00	Maternal distress NOS
L392.00	Maternal hypotension syndrome
L392000	Maternal hypotension syndrome unspecified
L392300	Maternal hypotension syndrome with antenatal problem
L392z00	Maternal hypotension syndrome NOS
L394.00	Other complications of obstetric procedures
L394000	Other complications of obstetric procedures unspecified
L394z00	Other complications of obstetric procedures NOS
L410500	Varicose veins of legs in pregnancy
L411500	Genital varices in pregnancy
L411511	Perineal varices in pregnancy
L411512	Vaginal varices in pregnancy
L411513	Vulval varices in pregnancy
L412500	Superficial thrombophlebitis in pregnancy
L412511	Thrombophlebitis of legs in pregnancy
L413.00	Antenatal deep vein thrombosis
L413.11	DVT - deep venous thrombosis, antenatal
L413000	Antenatal deep vein thrombosis unspecified
L413200	Antenatal deep vein thrombosis with antenatal complication
L413z00	Antenatal deep vein thrombosis NOS

L414.12	Phlegmasia alba dolens - obstetric
L415500	Other phlebitis in pregnancy
L416600	Haemorrhoids in pregnancy
L417000	Cerebral venous thrombosis in pregnancy
L41z500	Venous complication of pregnancy, unspecified
L431300	Amniotic fluid pulmonary embolism with a/n complication
L43y.11	Fat embolism - obstetric
L43y300	Other obstetric pulmonary embolism with antenatal comp
L4500F	CONTRACEPTION I U C D FAILURE
L465300	Suppressed lactation with antenatal complication
L46z300	Disorder of lactation NOS with antenatal complication
L5...00	Maternal care for fetus
L51..00	Maternal care for other known or suspected fetal problems
L510.00	Maternal care for hydrops fetalis
L511.00	Maternal care for viable fetus in abdominal pregnancy
L512.00	Maternal care for diminished fetal movements
L514.00	Maternal care for poor fetal growth
L51X.00	Maternal care/known or suspected fetal problem,unspecifd
L6000UP	PREGNANCY UNPLANNED
L9610UP	PREGNANCY UNWANTED
Lyu0100	[X]Other specified abnormal products of conception
Lyu0400	[X]Oth+unspcf failed inducd abort,complct gen tract+pelv inf
Lyu0500	[X]Oth+unspc fail induc abortn,complict/delay/exces h'morrhg
Lyu0600	[X]Other+unspcf failed induced abortion,complicated/embolism
Lyu0700	[X]Oth+unspcf failed inducd abortn,wth oth+unspcf complicatn
Lyu0800	[X]Other+unspcf failed induced abortion,without complication
Lyu2.00	[X]Other maternal disorders predominant related to pregnancy
Lyu2000	[X]Other haemorrhage in early pregnancy
Lyu2100	[X]Other vomiting complicating pregnancy
Lyu2200	[X]Other venous complications in pregnancy
Lyu2300	[X]Infections of other parts of urinary tract in pregnancy
Lyu2400	[X]Other+unspcf genitourinary tract infection in pregnancy
Lyu2500	[X]Other specified pregnancy-related conditions
Lyu2600	[X]Other abnormal findings on antenatal screening of mother

Lyu2A00	[X]Abnormal finding on antenatal screening of mother
Lyu3000	[X]Other multiple gestation
Lyu3100	[X]Other complications specific to multiple gestation
Lyu3300	[X]Maternal care for other abnormalities of cervix
Lyu3400	[X]Maternal care for other abnormalities of gravid uterus
Lyu3500	[X]Maternal care for other abnormalities of pelvic organs
Lyu3600	[X]Maternal care/(suspected)damage/fetus/oth medicl procedur
Lyu3700	[X]Maternal care/other(suspected)fetal abnormality+damage
Lyu3800	[X]Maternal care for other isoimmunization
Lyu3900	[X]Maternal care/oth spcf known or suspected fetal problems
Lyu3A00	[X]Maternal care/known or suspected fetal problem,unspecifd
Lyu3B00	[X]Other disorders of amniotic fluid and membranes
Lyu3C00	[X]Other placental disorders
Lyu3D00	[X]Other premature separation of placenta
Lyu3E00	[X]Other antepartum haemorrhage
M240500	Alopecia of pregnancy
Q000.11	Fetus affected by maternal toxaemia
Q007111	Fetal alcohol syndrome
Q013.11	Fetus affected by hydramnios
Q014100	Fetus or neonate affected by abdominal ectopic pregnancy
Q015100	Fetus or neonate affected by twin pregnancy
Q016.11	Fetus affected by maternal death
Q017.11	Fetus affected by malpresentation
Q02..00	Fetus/neonate affected by complic of placenta/cord/membrane
Q021000	Fetus/neonate affected by antepartum haemorrhage unspecified
Q021011	Fetus affected by APH - antepartum haemorrhage
Q021111	Fetus affected by placental abruption
Q021200	Fetus/neonate affected by placental damage-amniocentesis
Q021500	Fetus or neonate affected by premature placental separation
Q021511	Fetus/neonate affected-prem placental separation+acc haem'ge
Q021y00	Fetus/neonate affected placental separation/haemorrhage OS
Q021z00	Fetus/neonate affected placental separation/haemorrhage NOS
Q022.11	Fetus affected by placental insufficiency
Q026.11	Fetus affected by cord problems

Q10..00	Slow fetal growth and fetal malnutrition
Q10..11	Fetal malnutrition
Q100.00	Fetus small-for-dates, without mention of malnutrition
Q100.11	Fetus small-for-dates (SFD), without mention of malnutrition
Q101.00	Fetus small-for-dates with signs of malnutrition
Q101.11	Fetus small-for-dates (SFD) with signs of malnutrition
Q102.00	Fetal malnutrition, no mention light or small for gest age
Q102.11	Fetal malnutrition without mention of 'light for dates'
Q10z.00	Fetal growth retardation NOS
Q10z.11	Intrauterine growth retardation
Q21..11	Intrauterine hypoxia
Q210.00	Fetal death due to prelabour anoxia
Q408.00	Intra-amniotic fetal infection
Q408000	Intra-amniotic fetal infection, unspecified
Q408100	Clostridial intra-amniotic fetal infection
Q408200	Eschericha coli intra-amniotic fetal infection
Q408300	Staphylococcal intra-amniotic infection NEC
Q408500	Group B haemolytic streptococcal intra-amniotic infect. NEC
Q408z00	Intra-amniotic fetal infection NOS
Q40y000	Intrauterine fetal sepsis, unspecified
Q410.00	Fetal blood loss
Q410000	Fetal blood loss, unspecified
Q410200	Fetal placental blood loss
Q410400	Fetal blood loss from vasa praevia
Q410500	Fetal haemorrhage into co-twin
Q410600	Fetal haemorrhage into mother's circulation
Q410700	Fetal exsanguination
Q410y00	Other specified fetal blood loss
Q410z00	Fetal blood loss NOS
Q423.00	Hydrops fetalis due to isoimmunisation
Q42X.00	Hydrops fetalis due to other+unspcfd haemolytic disease
Q436.00	Fetal and neonatal jaundice, unspecified
Q454100	Polycythaemia due to maternal fetal transfusion
Q470.00	Idiopathic hydrops fetalis
Q479.00	Non-immune hydrops fetalis
Qyu0400	[X]Fetus+newbrn affect/oth forms/placental separatn+h'morrhg
Qyu0700	[X]Fetus+newborn affectd/oth+unspcfd conditns/umbilical cord
Qyu5000	[X]Other fetal blood loss
Qyu5600	[X]Hydrops fetalis due to other+unspcfd haemolytic disease
QyuA500	[X]Complications of intrauterine procedures NEC

T182	PREGNANCY HIGH RISK
T347	ADVICE GIVEN ON ABORTION
T349	REQUESTS ABORTION
T800	PREGNANCY OPERATION DURING
T901	PREGNANCY WITH I U D IN PLACE
T9011	IUCD FAILED
T961 AA	PREGNANCY UNMARRIED
T961 AC	PROBLEM PREGNANCY UNMARRIED
T961 AD	PROBLEM UNMARRIED PREGNANCY
T961 AE	PREGNANCY OUT OF WEDLOCK
T967	PROBLEM PREGNANCY
T976	TERMINATION REFUSED PREGNANCY
T9761	DECIDED AGAINST TERMINATION PREGNANCY
Y409 D	RUBELLA CONTACT IN PREGNANCY
Y409 DA	RUBELLA CONTACT IN EARLY PREGNANCY
Y60	PREGNANCY
Y60 A	PREGNANCY NORMAL
Y60 AA	PREGNANT
Y60 AM	INSTRUCTION ANTENATAL GIVEN
Y60 AN	INSTRUCTION ANTENATAL
Y60 B	PROPHYLACTIC THERAPY PREGNANCY
Y60 BA	PREGNANCY PROPHYLACTIC THERAPY PRESCRIBE
Y60 CA	PREGNANCY PRENATAL CARE
Y60 CB	PREGNANCY PRENATAL CARE NORMAL
Y60 EL	MEDICAL EXAMINATION ANTENATAL
Y60 EM	MEDICAL EXAMINATION PREGNANCY
Y60 EN	PREGNANCY BOOKING CONSULTATION
Y60 EP	PRENATAL EXAMINATION
Y60 EQ	PREGNANCY EXAMINATION NORMAL
Y60 ER	EXAMINATION PRENATAL
Y60 NA	ANTENATAL CARE
Y60 NB	ANTENATAL BOOKING
Y60 NC	BOOKING ANTENATAL
Y60 NP	PRENATAL CARE NORMAL PREGNANCY
Y60 NQ	PRENATAL CARE REGULARLY ATTENDED
Y60 NR	PREGNANCY ANTENATAL CARE NORMAL
Y60 NS	NORMAL PREGNANCY PRENATAL CARE THROUGHOU
Y601 HH	FOETAL HEART HEARD
Y601 HN	FOETAL HEART NORMAL
Y601 MF	FOETAL MOVEMENTS FELT
Y601 MN	FOETAL MOVEMENTS NORMAL
Y601 MS	FOETAL MOVEMENTS STOPPED
Z212.00	Antenatal care
Z212.11	Pregnancy care
Z212100	Delivery place planned
Z212200	Home delivery planned
Z212300	Delivery place booked
Z22..00	Pregnancy observations
Z221.00	Primigravida
Z222.00	Multigravida

Z225.00	Normal pregnancy
Z226.00	Pregnancy problem
Z227.00	Confirmation of pregnancy
Z229.00	Observation of position of pregnancy
Z229100	Intrauterine pregnancy
Z22A.00	Observation of pattern of pregnancy
Z22A100	Low risk pregnancy
Z22A200	High risk pregnancy
Z22A300	Concealed pregnancy
Z22A400	Early stage of pregnancy
Z22A500	Biochemical pregnancy
Z22A600	Teenage pregnancy
Z22A700	Surrogate pregnancy
Z22A800	Undiagnosed pregnancy
Z22A900	Unwanted pregnancy
Z22AA00	Wanted pregnancy
Z22AB00	Unplanned pregnancy
Z22AB11	Accidental pregnancy
Z22AC00	Pregnancy with uncertain dates
Z22AD00	Presentation of pregnancy
Z22AD11	Reported conception - pregnancy
Z22AE00	Baby overdue
Z22B.00	Observation of quantity of pregnancy
Z22B100	Single pregnancy
Z22B900	Continuing pregnancy after abortion of sibling fetus
Z22BA00	Contin pregnancy after intrauterine death of sibling fetus
Z22C.00	Observation of measures of pregnancy
Z22C100	Estimated date of delivery from last period
Z22C200	Estimated date of delivery from last normal period
Z22C300	Length of gestation
Z22C311	Pregnancy duration
Z22C312	Duration of gestation
Z22C313	Duration of pregnancy
Z22C314	Weeks pregnant
Z22C500	Estimated date of conception
Z22C511	EDC - Estimated date of conception
Z22CF00	Date symptom of pregnancy first noted
Z22D.00	Observation of viability of pregnancy
Z22D100	Viable pregnancy
Z22D200	Non-viable pregnancy
Z22D300	Uncertain viability of pregnancy
Z22D311	Query viability of pregnancy
Z23..00	Observation of gravid uterus
Z231.00	Gravid uterus present
Z234.00	Observation of size of gravid uterus
Z234100	Gravid uterus large-for-dates
Z234200	Gravid uterus small-for-dates
Z234300	Observation of height of gravid uterus
Z234400	Fundal height high for dates

Z234500	Fundal height equal to dates
Z234600	Fundal height low for dates
Z235.00	Observation of shape of pregnant abdomen
Z235300	Transversely enlarged pregnant abdomen
Z235400	Pendulous pregnant abdomen
Z236100	Normal position of gravid uterus
Z237.00	Observation of sensation of gravid uterus
Z237200	Tender scar of gravid uterus
Z238112	Fundus firm
Z239200	Uterine contractions absent
Z239300	Uterine contractions ceased
Z239800	Uterine activity
Z23A100	Intermittent uterine contractions
Z23A200	Occasional uterine tightenings
Z23A400	Irregular uterine contractions
Z23A700	Niggling uterine contractions
Z23A800	Mild uterine contractions
Z23AO00	Irritable uterus
Z23AP00	Premature uterine contraction
Z23B100	Date false contractions first detected
Z23D100	Girth of pregnant abdomen
Z23D200	Pregnant abdomen observation
Z23E.00	Gravid uterus normal
Z23F.00	Gravid uterus problem
Z242.00	Labour not established
Z243200	First stage of labour not established
Z245100	Maternal blood loss minimal
Z245400	Maternal blood loss heavy
Z246500	Time vaginal show detected
Z252.00	Mother not delivered
Z26..00	Observation of structures of conception
Z261.00	Observation of gestational sac
Z261100	Gestational sac present
Z261200	Gestational sac absent
Z262C00	Retroplacental clot
Z262D00	Fresh retroplacental clot
Z262E00	Old retroplacental clot
Z262E11	Stale retroplacental clot
Z262J11	Placental infection
Z262R00	Placenta problem
Z263.00	Uterine membrane observations
Z263900	Number of chorions in membranes
Z263C00	Intact membranes
Z264100	Observation of quantity of liquor
Z264111	Quantity of liquor
Z264112	Amount of liquor
Z264400	Normal liquor volume
Z264500	Reduced amniotic fluid
Z264600	Excessive amniotic fluid
Z265.00	Umbilical cord observations

Z265200	Umbilical cord problem
Z265A00	Number of blood vessels in umbilical cord
Z265A11	Number of vessels entering umbilical cord
Z265B00	Number of umbilical arteries
Z265C00	Number of umbilical veins
Z271.00	Cardiotochogram observation
Z271.11	CTG observations
Z271100	Fetal heart acceleration
Z271200	Normal CTG tracing
Z271400	Unsatisfactory CTG tracing
Z271411	Technically poor CTG
Z271500	CTG reactivity
Z271600	Reactive CTG tracing
Z271900	Fetal heart rate variability
Z271912	FHRV - Fetal heart rate variability
Z271C00	Fetal heart baseline pattern
Z271D00	Normal fetal heart baseline pattern
Z271E00	Sinusoidal pattern of fetal heart
Z271F00	Fetal heart deceleration
Z28..00	Obstetric pelvic observation
Z28..11	Pelvic assessment - childbirth
Z282300	Flat sacral curve
Z288.00	Problem of pelvis for delivery
Z675.00	Antenatal class
ZL75.00	Referral to midwife
ZV22.00	[V]Normal pregnancy
ZV22.11	[V]Supervision of normal pregnancy
ZV22000	[V]First normal pregnancy supervision
ZV22100	[V]Other normal pregnancy supervision
ZV22200	[V]Pregnancy confirmed
ZV22300	[V]Pregnant state, incidental
ZV22400	[V]Supervision of other normal pregnancy
ZV22y00	[V]Other specified pregnant state
ZV22z00	[V]Unspecified pregnant state
ZV23.00	[V]High-risk pregnancy supervision
ZV23000	[V]Pregnancy with history of infertility
ZV23100	[V]Pregnancy with history of trophoblastic disease
ZV23111	[V]Pregnancy with history of hydatidiform mole
ZV23112	[V]Pregnancy with history of vesicular mole
ZV23200	[V]Pregnancy with history of abortion
ZV23400	[V]Pregnancy with other poor obstetric history
ZV23500	[V]Pregnancy with other poor reproductive history
ZV23600	[V]Supervisn/pregnancy wth history insufficnt antenatal care
ZV23800	[V]Supervision of high-risk pregnancy due to social problems
ZV23y00	[V]Other specified high-risk pregnancy
ZV23z00	[V]Unspecified high-risk pregnancy
ZV28.00	[V]Antenatal screening
ZV28y00	[V]Other specified antenatal screening

ZV4J000	[V]Problems related to unwanted pregnancy
ZV61800	[V]Illegitimate pregnancy
ZV61900	[V]Other unwanted pregnancy
ZVu2300	[X]Supervision of other normal pregnancy
l413.00	Antenatal deep vein thrombosis