Understanding the Relationship Between Selenium, Iodine and Associated Biomarkers During Pregnancy and The Effect on Birth Weight

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Selenium and iodine are essential dietary trace elements required for oxidative protection and thyroid hormone metabolism. Iodine deficiency states have been linked to serious clinical complications in pregnancy for both mother and child, however current assessments of selenium status in the UK pregnant population remains limited. The present study aims to assess selenium and iodine concentrations during pregnancy in a UK based cohort, investigating for the first time potential associations between these two micronutrients. Serum selenium, urinary iodine, serum GPx3, serum SEPP1, serum free triiodothyronine (fT3), serum free thyroxine (fT4), serum TSH and serum thyroglobulin was measured in 70 prospective mothers from the Health & Iodine Status in Babies (HIBA) cohort. Serum samples were collected at three time points spanning gestation (~13-15 weeks, ~24 weeks and ~36 weeks) and quantified using ICP-MS/ELISA methodologies. Longitudinal assessments were performed to monitor how concentrations changed over time. Serum selenium concentration was associated with iodine, relevant biomarkers, dietary intake and birthweight using repeat measure linear regression. Median (interquartile range) serum selenium concentrations across the three time-points of pregnancy were 71.4(16.4), 64.2(12.2) and 57.7(15.3) µg/L, respectively. Adjusted linear regression analysis found significant associations between selenium and GPx3 (p<0.001), SEPP1 (p<0.001) and birthweight (p=0.02, first time point), however no associations between diet and iodine status were observed. This study found that the mothers in this cohort were generally selenium deficient as per recommended guidelines (90-100 µg/L). For the first time within a UK population, selenium concentration was found to be associated with birthweight during early pregnancy with a 10 µg increase in serum selenium concentration leading to a 150 g rise in birthweight. As such, the results of this study could have a major impact on the nutritional guidance recommended to UK mothers, though further research with a larger population is warranted to consolidate findings.

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µg	Microgram
µIU	Micro international units
µL	Microlitre
µmol	Micromolar
AI	Adequate intake
ATP	Adenosine triphosphate
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CHD	Coronary heart disease
CI	Confidence interval
cm	Centimetre
COMA	Committee on Medical Aspects of Food and Nutrition Policy
CRM	Certified reference material
CVD	Cardiovascular disease
DIO	Iodothyronine deiodinase
DIO1	Iodothyronine deiodinase 1
DIO2	Iodothyronine deiodinase 2
DIO3	Iodothyronine deiodinase 3
DIT	Diiodotyrosine
DRV	Dietary reference value
EFSec	Specialised elongation factor
ELISA	Enzyme linked immunoassay
EQUIP	Ensuring the Quality of Urinary Iodine Procedures
EU	European Union
fT3	Free 3,5,3'-triiodothyronine
fT4	Free 3,5,3',5'-tetraiodothyronine

g	Gram
GPx	Glutathione peroxidase
GPx1	Glutathione peroxidase 1
GPx2	Glutathione peroxidase 2
GPx3	Glutathione peroxidase 3
GPx4	Glutathione peroxidase 4
GPx6	Glutathione peroxidase 6
GSH	Glutathione
GS-Se-SG	Selenodiglutathione
GSSG	Glutathione disulfide
H_2O	Water
H_2O_2	Hydrogen peroxidase
H_2Se	Hydrogen selenide
hCG	Human chorionic gonadotrophin
HIBA	Health & Iodine Status in Babies
HPT	Hypothalamic-pituitary
HRP	Horse radish peroxidase
$HSePO_3^{2-}$	Selenophosphate
ICCIDD	International Council for Control of Iodine Deficiency Disorders
ICP-MS	Inductively coupled plasma mass spectrometry
IDD	Iodine deficiency disorder
IoM	United States Institute of Medicine
IQR	Interquartile range
KDa	Kilo Dalton
kg	Kilogram
m/z	Mass-to-charge ratio
mg	Milligram
MI	Myocardial infarction

MIT	Monoiodotyrosine
mL	Millilitre
mRNA	Messenger ribonucleic acid
NADPH	Dihydronicotinamide-adenine dinucleotide phosphate
NDNS	National Diet and Nutrition Survey
ng	Nanogram
nm	Nanometre
pg	Picogram
ppb	Parts per billion
PVE	Plasma volume expansion
QC	Quality control
RDA	Recommended daily allowance
RNI	Reference nutrient intake
ROS	Reactive oxidative species
rT3	3,3’,5’-triiodothyronine (reverse triiodothyronine)
SACN	Scientific Advisory Committee of Nutrition
SBP2	Selenocysteine insertion sequence (SECIS) -binding protein 2
SD	Standard deviation
Sec	Selenocysteine
SECIS	Selenocysteine insertion sequence
SeMet	Selenomethionine
SeOH	Selenic acid
SEPP1	Selenoprotein P
Ser	Serine
T2	3,5-diiodo-L-thyronine
T3	3,5,3'-triiodothyronine
T4	3,5,3',5'-tetraiodothyronine
TBG	Thyroxine binding globulin

TMB	Tetramethylbenzidine
TPO	Thyroid peroxidase
TRH	Thyrotropin releasing hormone
tRNA	Transfer ribonucleic acid
TrxR1	Thioredoxin reductase 1
TrxR2	Thioredoxin reductase 2
TrxR3	Thioredoxin reductase 3
TSH	Thyroid stimulating hormone
UIC	Urinary iodine concentration
UK	The United Kingdom of Great Britain and Northern Ireland
UK NEQAS	United Kingdom National External Quality Assessment Service
UNICEF	United Nations International Children's Emergency Fund.
USA	United States of America
USI	Universal salt iodisation
WHO	World Health Organization

Introduction

A balanced, nutritious diet is crucial for optimal micronutrient intake (National Health Service, 2019). Micronutrients play key roles within metabolism and are essential for normal biological processes and the aversion of disease (Shenkin, 2006). Specific requirements for vitamins, minerals and trace elements for population groups have been recommended by independent organisations and government public health bodies such as the World Health Organisation (WHO) and the United Kingdom of Great Britain and Northern Ireland (UK) Scientific Advisory Committee of Nutrition (SACN) (World Health Organisation, 2004; Public Health England, 2016a).

Additional nutritional requirements are often necessary in certain vulnerable groups including expectant mothers (Shenkin, 2006). Pregnancy is a time of substantial physiological and metabolic change with increased nutritional demands essential to sustaining normal maternal homeostasis and foetal development (Mousa et al., 2019). Sufficient intake of micronutrients such as iron, vitamin A, folate, selenium and iodine are necessary throughout gestation to meet these pregnancy requirements (Black, 2007). Micronutrient inadequacy can therefore result in maternal deficient states which carries the potential for serious complications in both mother and foetus (Mousa et al., 2019). The effects of severe micronutrient deficiencies during gestation are well established. For example, extensive research has proven that insufficient folate intake both prior to and during pregnancy can result in incomplete neural-tube formation during the first 28 days of pregnancy (Medical Research Council Vitamin Study Research Group, 1991; Berry et al., 1999). This manifests as debilitating neural tube defects including spina bifida and anencephaly (Gernand et al., 2016). To prevent these adverse birth outcomes, the UK government recommends folic acid supplementation 3 months before conception and throughout the first trimester up to 12 weeks (National Health Service, 2018).

In western societies, severe micronutrient deficiencies are less common compared to that of the developing world. This is attributed to higher socio-economic status, increased access to supplementation and the widespread intake of fortified foods (Gernand et al., 2016). However, mild to moderate deficiencies in some micronutrients are becoming increasingly more apparent in some western demographic areas. This is thought to be the consequence of dietary choices i.e., vegetarianism (Allen, 2005), the ease of accessibility to fatty/sugary foods resulting

in the reduced consumption of nutritionally rich items (Gernand et al., 2016) and the geochemical conditions of particular continents and countries (Johnson et al., 2010).

Iodine is a micronutrient thought to be mildly deficient within the UK pregnant population (Bath et al., 2014; Furmidge-Owen et al., 2014). This is reflected within the latest National Diet and Nutrition Survey (NDNS) showing that 17% of women at child bearing age were iodine deficient according to the WHO guidelines (Public Health England, 2018). Essential to mammalian existence (Zimmermann, 2009a), iodine is the key constituent of the two major thyroid hormones; 3,5,3'-triiodothyronine (T3) and 3,5,3',5'-tetraiodothyronine/thyroxine (T4) (SACN, 2014). T3 and T4 influence the majority of cells within the human body via their effects on neuronal maturation and basal metabolic rate (Nussey and Whitehead, 2001). Iodine insufficiency throughout pregnancy has been linked to adverse birth outcomes such as impaired foetal brain development, low intelligence and impaired motor skills (Rayman et al., 2009). In conjunction with iodine, selenium is another essential micronutrient pivotal for the production of vital selenoproteins including selenoprotein P (SEPP1), glutathione peroxidase 3 (GPx3) and iodothyronine deiodinases (DIOs) (Wrobel et al., 2016). These selenium containing proteins are implicated in selenium transport (Ventura et al., 2017), antioxidative defence (Wu et al., 2015) as well as thyroid hormone metabolism (Zimmermann and Kohrle, 2002). Selenium status and its effects during pregnancy are poorly recognised compared to other important micronutrients such as iodine. Therefore, the aim of this study is to investigate the role of both these essential trace elements throughout the course of pregnancy.

1.1 Selenium

1.1.1 Background

Selenium (chemical symbol: Se), was discovered by chemist Jacob Berzelius and Johan Gottlieb Gahn in 1817, however it was not linked with human health until 150 years after its initial discovery. The work of Schwarz and Foltz (1957) first showed the importance of dietary selenium, demonstrating that liver necrosis could be prevented by feeding rodents yeast with selenium-containing Factor 3. As a relatively new discovery in biological terms, the effect of selenium and its incorporation into proteins via the essential amino acid selenocysteine (Sec) has started to be linked with thyroid function, cardiovascular disease and immune function (Beckett and Arthur, 2005; Boyd, 2011). These links with human health have therefore cemented selenium as an essential trace element required for mammalian life (Combs, 2001).

1.1.2 Selenium Geochemistry

Selenium resides naturally in the earth's crust averaging concentrations of ~0.01-2 mg/kg (Thiry et al., 2012). Existing in both organic (selenomethionine [SeMet]$^{-2}$ and Sec^{-2}) and inorganic forms (selenium selenate^{+6}, selenium selenide^{-2} and selenium selenite^{+4}) (Mehdi et al., 2013), selenium levels in the environment are predominantly influenced by soil properties, bioavailability and geographical location (Navarro-Alarcon and Cabrera-Vique, 2008; Hartikainen, 2005). Selenium soil content is determined by a multitude of factors such as rock type, pH, rainfall, soil fertilisation, selenium speciation and sulphate concentration (Navarro-Alarcon and Cabrera-Vique, 2008). Sedimentary rock has higher selenium concentrations compared to volcanic rock, limestone and sandstone which are commonly found in mountainous areas of Europe i.e. Scotland, Finland and Sweden (Mehdi et al., 2013; Johnson et al., 2010). pH also has a substantial effect on the bioavailability of selenium. Alkaline conditions tend to favour selenium selenate^{+6}. This makes it more soluble within the soil environment, resulting in unimpaired plant uptake. In comparison, acidic conditions favour selenium selenite^{+4}. Selenite's strong adsorption to clay, forming chemical oxides reduces the selenium solubility (Combs, 2001; Navarro-Alarcon and Cabrera-Vique, 2008). Additionally, rainfall and soil moisture can directly lower plant selenium bioavailability via leaching; selenium bioavailability is more profound in drier conditions (Combs, 2001). Selenium fertilisers have the ability to drastically alter soil selenium concentrations. For example, sodium selenate has been used to increase selenium concentration in agricultural produce such as cereal (Hartikainen, 2005). Lastly, soil sulphur concentrations can also widely effect selenium uptake in plants. Residing in the same elemental family, sulphur and selenium exhibit similar properties such as atomic size, oxidation states and ionisation potentials (Mehdi et al., 2013). As a result, the antagonistic effect of sulphur can negatively affect selenium uptake through plants as they both require use of the same sulphate transporters (Terry et al., 2000).

The combination of these factors means that global selenium distribution ranges extensively. Selenium replete soils are found between geographical areas such as the Americas, Canada and Japan (Schomburg and Kohrle, 2008), with less replete soils commonly found in China, Finland and Denmark (Combs, 2001). Certain parts of the UK i.e. Wales has a variable geographical distribution of selenium as a result of its diverse geology. For example, rocks and soils in areas such as Snowdonia and the Parys Mountain range are typically high in selenium. This is in contrast to the lower sandstone areas of mid-Wales where lower selenium concentrations are common (Johnson et al., 2010).

1.1.3 Dietary Sources of Selenium

The major source of selenium is derived from the diet, with concentrations being dependent on the food type and the quantity consumed (Navarro-Alarcon and Cabrera-Vique, 2008). Table 1 shows the average selenium content of foods within the UK (SACN, 2013). Cereal grain, fish, and meat produce account for the highest intakes of selenium as defined by the latest NDNS Survey Years 7&8 (Public Health England, 2018). Brazil nuts contain large concentrations of selenium, however they are not consumed frequently within UK populations (Santhosh-Kumar and Priyadarsini, 2014). Conversely, fruit and vegetables are relatively low in selenium content, providing only small amounts in the human diet (Combs, 2001). Skeletal muscle is generally low in selenium concentration, however organ meats such as liver and kidney can contain on average up to 4-5 times more selenium (as seen in Table 1) (Combs, 2001). Dietary selenium intake varies widely as a consequence of geochemical (see section 1.1.2), economic and processing factors. Trade and the importation of selenium rich food items from replete countries such as Canadian cereal grain, has been shown to raise selenium intake within the UK population (MacPherson et al., 1997). Certain cooking processes have also been shown to alter selenium content. For example, the boiling of certain foods such as asparagus sees selenium concentration drop by as much as 40% (Navarro-Alarcon and Cabrera-Vique, 2008). As a result, selenium food tables are commonly imprecise (Combs, 2001).

Food	Mean selenium content (µg/100g)	Food	Mean selenium content (µg/100g)
Brazil Nuts, Kernel	85-690	Salmon*	19.5
Kidney*	250	Chicken*	16
Canned Tuna^	87	Beef*	10
Liver*	62	Wholemeal Bread	7
Cod*	44	White Bread	6
Prawns*	30	Cornflakes	5
Egg*	27	Lamb*	4

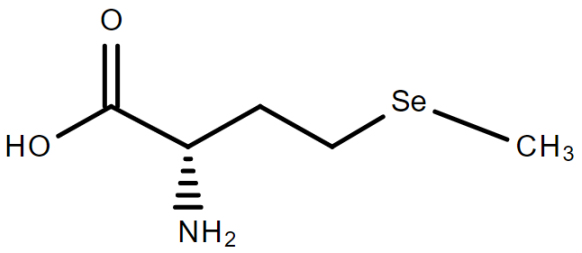
Table 1: Selenium content of UK food sources. The table displays selenium rich foods within the UK and the estimated selenium content (µg/100g). *selenium content after cooking. Table adopted from SACN (2013).

1.1.4 Bioavailability and Metabolism

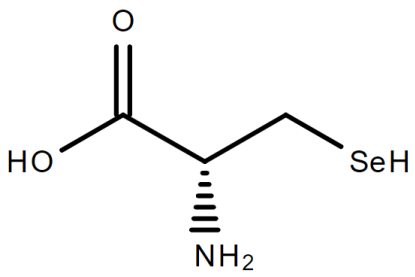
1.1.4.1 Selenium Bioavailability

Four selenium species account for the majority of dietary selenium intake; SeMet, Sec, selenium selenite and selenium selenate (Burk and Hill, 2015). Absorption of selenium species occurs in the lower intestinal tract through different routes and mechanisms, some of which are still yet to be fully characterised (Fairweather-Tait et al., 2010). Originating from plant and animal sources, SeMet and Sec (Figure 1) are both readily absorbed by the small intestine via the same sodium-dependant transcellular absorption pathways like that of their sulphur-containing analogues (Roman et al., 2014). This occurs due to selenium and sulphur having similar chemical properties (discussed in section 1.1.2) (Navarro-Alarcon and Cabrera-Vique, 2008). Inorganic selenium selenate is para-cellularly absorbed into the body via a passive diffusion process, however the absorption process of selenium selenite is still yet to be determined (Roman et al., 2014).

Overall, the bioavailability of all selenium species is relatively high under normal, physiological conditions (~60-90%) (Finley, 2006). However, a number of factors have been shown to effect this value including the composition of specific food sources (fish and animal meats) and the presence of soil sulphur, certain vitamins and heavy metals (Thiry et al., 2012).



(A)



(B)

Figure 1: SeMet and Sec chemical structures. Chemical formulae of the two main organic selenium species; (A) SeMet (B) Sec. Adopted from the Royal Society of Chemistry (2015)

1.1.4.2 Selenium Metabolic Pathway

Selenium metabolism a complex process (Navarro-Alarcon and Cabrera-Vique, 2008). Present knowledge of human selenium metabolism is inferred from rat and mouse studies and is depicted in Figure 2 (Rayman, 2008).

Metabolic pathways are partially dependent on the species of selenium consumed (Finley, 2006). Organic SeMet is catabolised to Sec via the trans-selenation pathway. Sec generated from this pathway or yielded directly from the diet results in the production of hydrogen selenide (H_2Se) via catalysis from Sec β-lyase (Rayman, 2008). SeMet has the added functionality of being non-specifically inserted into methionine positions during protein synthesis (Fairweather-Tait et al., 2010), occurring via the methionine cycle/trans-sulfuration pathway (Lajin et al., 2016). This incorporation can prove to be an important unregulated pool of selenium during deficient states (Wrobel et al., 2016). The absorption of inorganic selenium utilises the reductive metabolic pathway to produce the same common precursor, H_2Se (Finley, 2006). Selenium selenate is firstly reduced to selenium selenite by adenosine triphosphate (ATP)-sulfurylase (Roman et al., 2014). Further downstream reduction of selenium selenite to selenodiglutathione (GS-Se-SG) catalysed by dihydronicotinamide-adenine dinucleotide phosphate (NADPH) dependant reductases and glutathione reductases leads to H_2Se formation (Wrobel et al., 2016). As the preferred precursor for selenoprotein production (Fairweather-Tait et al., 2010), H_2Se is used for downstream metabolism of selenophosphate ($HSePO_3^{2-}$); the selenium donor for selenoprotein synthesis (section 1.1.4.3) (Wrobel et al., 2016). Excess concentrations of H_2Se are commonly excreted via the urine in the form of selenosugars or in the breath (Lajin et al., 2016).

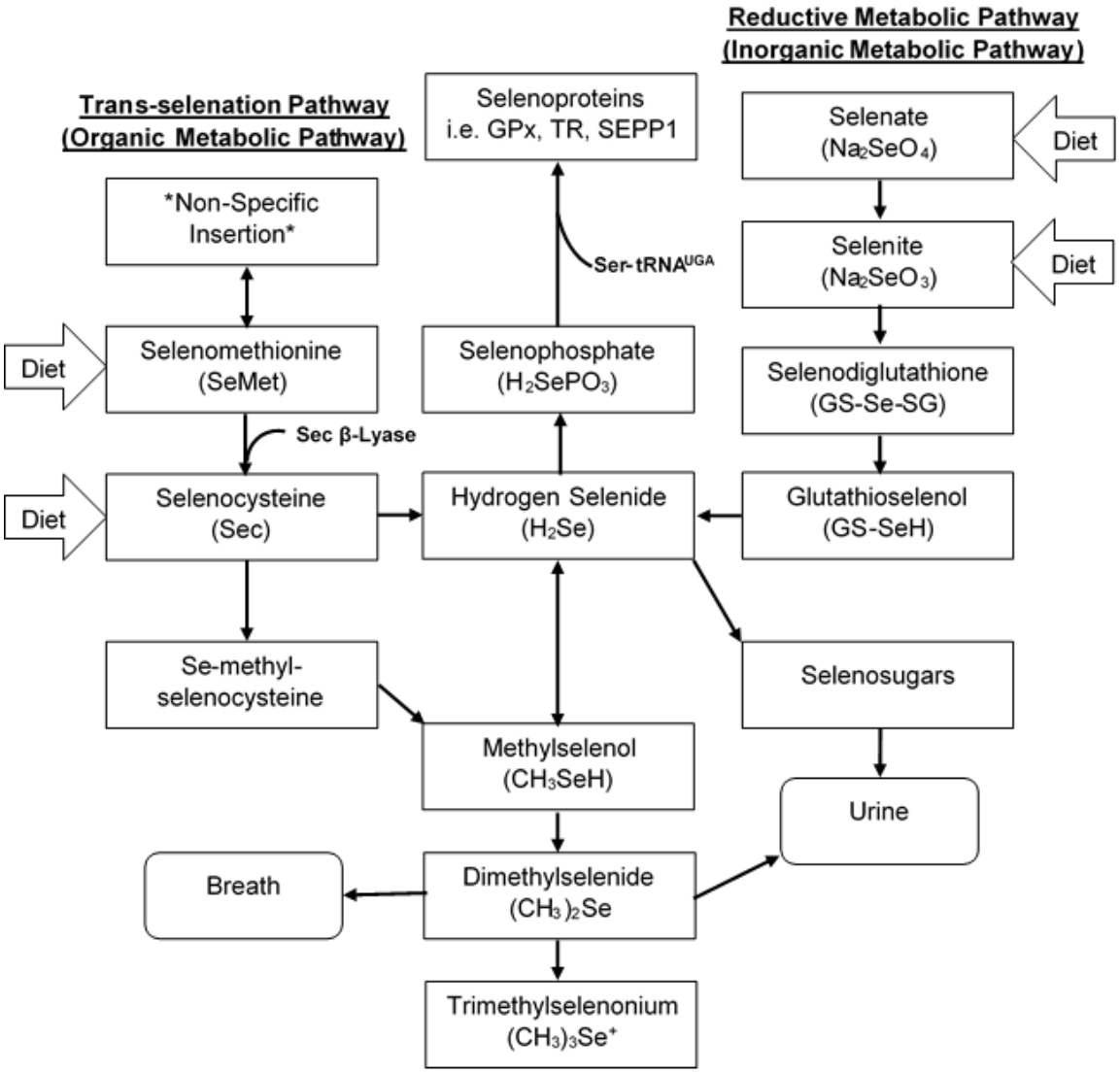


Figure 2: Metabolic pathway of selenium. Schematic representation of dietary selenium metabolism. After selenium is absorbed in the lower intestinal tract, SeMet is transported to the liver in the form of selenoalbumin whilst the remaining selenium species are transported either intact or through other mechanisms not currently established (Roman et al., 2014). This is the primary site of selenium metabolism since the majority of selenoproteins are translated and expressed in this organ. The reductive pathway (depicted on the right hand side) outlines the metabolic pathway that selenium selenate and selenium selenite utilises to produce H_2Se (described in main text). Organic selenium metabolism is depicted on the left hand side of the diagram. SeMet either becomes incorporated non-specifically into proteins via the trans-sulfuration pathway or is catabolised to Sec through the trans-selenation pathway. Both organic and inorganic metabolic pathways result in the same common precursor, H_2Se. Selenium regulation and excretion is also controlled by the liver. The organ has the ability to regulate whole body selenium by secreting transport selenoproteins into the plasma for use by other tissues (Burk and Hill, 2015). In periods of saturation, excess selenium is excreted through the metabolic pathways shown above. These processes involve the methylation to either a selenosugar from H_2Se or via further downstream methylation to dimethylselenide where selenium is exhaled in the breath (Rayman, 2008). Figure adapted from Wrobel et al. (2016), Rayman (2008) and Combs (2001).

1.1.4.3 Selenoprotein Synthesis

Selenoprotein synthesis is complex process involving specialised reactions leading to the production and insertion of a Sec amino acid (Figure 3) (Wrobel et al., 2016). Five main molecular components are utilised: selenoprotein messenger ribonucleic acid (mRNA), selenocysteyl—transfer ribonucleic acid (tRNA)$^{[ser]sec}$, a specialised translational elongation factor (EFSec), a stem and loop structure called the selenocysteine insertion sequence (SECIS) and the SECIS binding protein 2 (SBP2) (Drutel et al., 2013; Labunskyy et al., 2014)

Sec is the only known amino acid whereby biosynthesis occurs on its own, specialised tRNA (selenocysteyl-tRNA$^{[ser]sec}$). As one of the largest eukaryotic tRNA molecules at 90 nucleotides in length (Schomburg and Schweizer, 2009), it is formed from precursors derived from the selenium metabolic pathway (Figure 2). Firstly, tRNA$^{[ser]Sec}$ undergoes aminoacylation with serine which is then phosphorylated to produce O-phosphoseryl-tRNA$^{[ser]sec}$ (Roman et al., 2014). The active selenium precursor, $HSePO_3^2$ (catalysed by Sec synthase) (Labunskyy et al., 2014) then replaces the phosphoryl moiety of the tRNA to produce selenocysteyl-tRNA$^{[ser]sec}$ (Brigelius-Flohe and Maiorino, 2013). Sec incorporation is coded by the ‘UGA’ codon in the open reading frame within the selenoprotein mRNA. Since the stop codon ‘UGA’ normally signals translation termination, the stem-loop structure at the 3’ untranslated region of the selenoprotein mRNA (SECIS) dictates the correct translation of the ‘UGA’ to Sec by the selenocysteyl-tRNA$^{[ser]sec}$ (Wrobel et al., 2016). SECIS also recruits two trans-acting factors which are required for efficient recognition of the ‘UGA’ codon; SBP2 and EFSec (Drutel et al., 2013; Labunskyy et al., 2014). The interactions of both of these elements recruit selenocysteyl-tRNA$^{[ser]sec}$ and facilitates the incorporation of Sec into the forming polypeptide (Labunskyy et al., 2014).

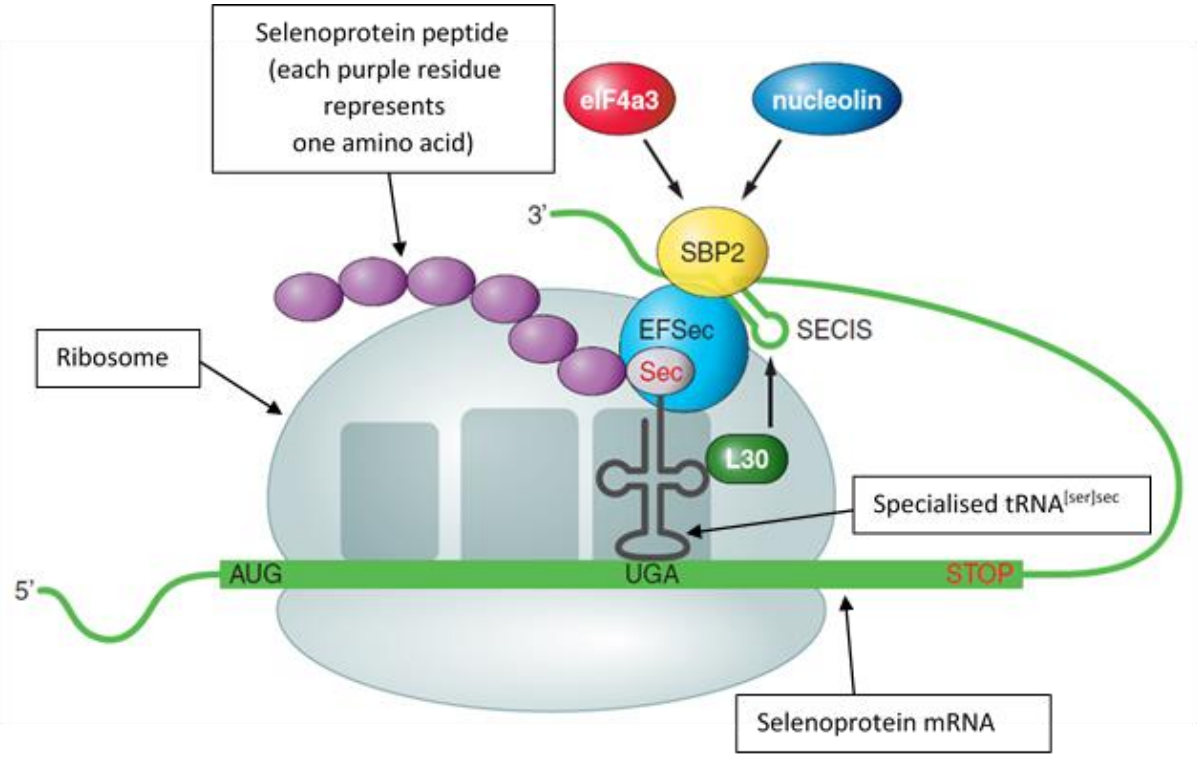


Figure 3: Eukaryotic Sec insertion. Machinery involved in the incorporation of a Sec molecule into a forming selenoprotein. Normal ribosomal translation of a 'UGA' codon would result in polypeptide termination, however the presence of the SECIS at the 3' untranslated region of the mRNA dictates that the 'UGA' codon should be recoded as a Sec molecule (Wrobel et al., 2016). In response, the specialised selenocysteyl -tRNA$^{[ser]sec}$ translates 'UGA' as Sec. Two main trans-acting factors; SBP2 and EFSec are essential to this process. SBP2 contains a specific RNA binding domain that binds SECIS elements with a high affinity. In addition, SBP2 also recruits EFSec which signals selenocysteyl-tRNA$^{[ser]sec}$ to the site of translation, therefore allowing Sec to be incorporated into the forming selenoprotein. Eukaryotic initiation factor 4a3 (eIF4a3), ribosomal protein L30 and nucleolin also serve as further SECIS-binding proteins. Whilst L30 aids Sec insertion, eIF4a3 and nucleolin act as regulatory proteins, modulating selenoprotein synthesis (Labunskyy et al., 2014). Adopted from Labunskyy et al. (2014).

1.1.5 Selenoproteins

Selenium exerts its biochemical function at the active site of selenoproteins via the Sec residue (Thiry et al., 2012; Duntas and Benvenga, 2015). Known as the "21st amino acid", Sec is an extremely effective biological redox catalyst implicated in important enzymatic functions such as thyroid hormone metabolism, fertility, redox reactions and lipid peroxidation (Brown and Arthur, 2007; Rayman, 2000; Ashton et al., 2009). Glutathione peroxidase 1 (GPx1) was the first selenoprotein to be discovered in 1973. Subsequently, a further 29 human selenoproteins encoded by 25 human genes make up the current human selenoproteome (Labunskyy et al., 2014). The main selenoproteins families implicated in thyroid homeostasis (Ventura et al., 2017) are described in Table 2.

Selenoprotein Family	Localisation		Key Functions
Glutathione Peroxidase 1, 2, 3, 4 and 6 (GPx1, GPx2, GPx3, GPx4, GPx6)	GPx1:	Cytosolic	Antioxidative function. Catalysing the reduction of peroxidases
	GPx2:	Gastrointestinal	
	GPx3:	Plasma	
	GPx4:	Phospholipid	
	GPx6:	*Unknown*	
Selenoprotein P (SEPP1)	Plasma, endothelial cells.		Selenium transport Antioxidative properties
Thioredoxin Reductase 1, 2 and 3 (TrxR1, TrxR2, TrxR3)	TrxR1:	Cytosolic	NADPH dependant reduction of thioredoxin for DNA synthesis.
	TrxR2:	Liver, kidney heart (mitochondria).	
	TrxR3:	Testis	
Iodothyronine Deiodinase 1, 2 and 3 (DIO1, DIO2, DIO3)	DIO1:	Thyroid, liver, kidney	Activation/deactivation of thyroid hormones
	DIO2:	Thyroid, brain, skeletal muscle	
	DIO3:	Placenta, brain	

Table 2: Key selenoprotein families involved in thyroid hormone metabolism. Localisation and general function of the four selenoprotein families required for thyroid homeostasis. Adapted from Rayman (2000), Rayman (2012), Ventura et al. (2017) and Moghadaszadeh and Beggs (2006).

1.1.5.1 Glutathione Peroxidase

Glutathione peroxidases (GPx) are a large family of antioxidant enzymes. There are five main human selenocysteine containing GPx's: GPx1, GPx2, GPx3, GPx4 and GPx6, all of which are involved in the reduction of hydrogen peroxide (H_2O_2) using glutathione (GSH) as the reductant cofactor (Brigelius-Flohe and Maiorino, 2013). This is achieved via a three-step pathway as seen in Figure 4.

GPx3 is a glycosylated tetrameric enzyme with a molecular weight of 93 kilo Dalton (KDa). It is the only extracellular GPx, being distributed within the plasma (Roman et al., 2014). It was first found in the proximal convoluted tubules of the kidneys where it is basolaterally secreted into plasma, however it has since been found in other tissues such as the thyroid gland (Brigelius-Flohe and Maiorino, 2013). Here it is secreted at the apical side of the thyrocyte membrane and is involved with the process of converting excess H_2O_2 after the iodination of tyrosyl residues into water (H_2O) (Rayman, 2018). GPx3 is sensitive to the concentration of whole body selenium with deficiency potentially leading to an impairment of antioxidant activity within the thyroid (Schomburg, 2011). As 90% of all GPx produced is that of GPx3, it makes it a good biomarker of overall selenium status (Burk and Hill, 2015).

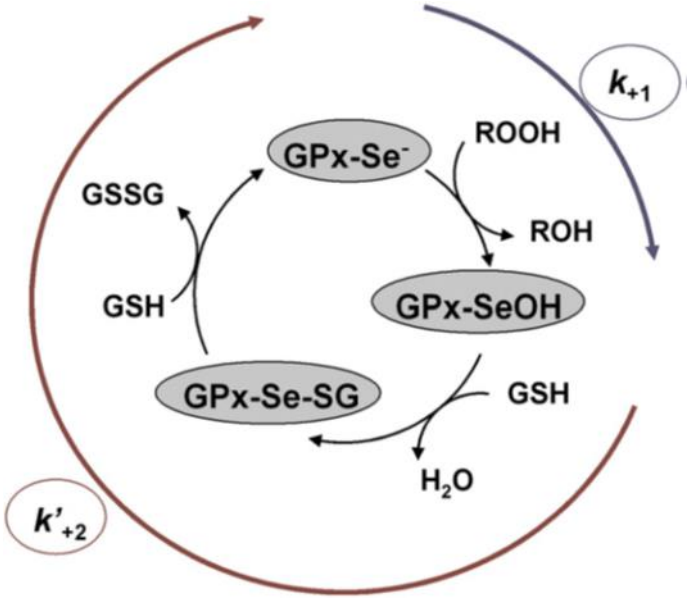


Figure 4: GPx oxidative pathway. A schematic diagram showing the GPx oxidation/reduction pathways. This has been proven for GPx1, GPx3 and GPx4. The selenol residue (GPx-Se^-) is oxidised by a hydroperoxide (ROOH) to form selenic acid (GPx-SeOH) (Roman et al., 2014). To regenerate the enzyme back to its selenol form, two molecules of GSH are reduced. The first GSH forms a selenadisulfide (GPx-Se-SG), with the second GSH reducing GPx-Se-SG to form a glutathione disulfide (GSSG) by-product and a regenerated selenol residue (Brigelius-Flohe and Maiorino, 2013). Figure adopted from Brigelius-Flohe and Maiorino (2013)

1.1.5.2 Selenoprotein P

SEPP1 consists of two main domains: the N- and C- terminals. In humans, the larger N-terminal domain contains one highly conserved Sec residue whereas the C-terminal domain contains the remaining nine. With a total of 10 Sec residues, SEPP1 is the highest selenium containing glycoprotein in the entire human body (Burk and Hill, 2009).

SEPP1 is synthesised in most tissues as seen by the presence of SEPP1 mRNA, however it is rapidly produced and exported in large quantities from the liver. SEPP1's main function is to act as a selenium transporter to extra-hepatic tissues such as the brain, testis and kidneys (Burk and Hill, 2005). For example, SEPP1 is bound by a lipoprotein receptor in the testis, facilitating the uptake of selenium into spermatozoa (Burk and Hill, 2009). In mouse models, selenium concentrations in the brain are diminished in the presence of SEPP1 gene deletion, showing that SEPP1 is crucial for selenium transportation (Hill et al., 2003). Studies have also suggested that SEPP1 exhibits some antioxidative properties (Shetty and Copeland, 2018). The 'UxxC' portion of the protein (see Figure 5) contains several conserved sequences similar to thioredoxin-fold proteins. This discovery suggests that the N-terminal domain shows thiol-redox function, owing to potential antioxidative properties (Burk and Hill, 2009). SEPP1 is the preferred biomarker of choice when assessing selenium status as over 50% of all plasma selenium is contained in SEPP1. This makes SEPP1 concentration a good biomarker selenium status within a population (Mostert, 2000).

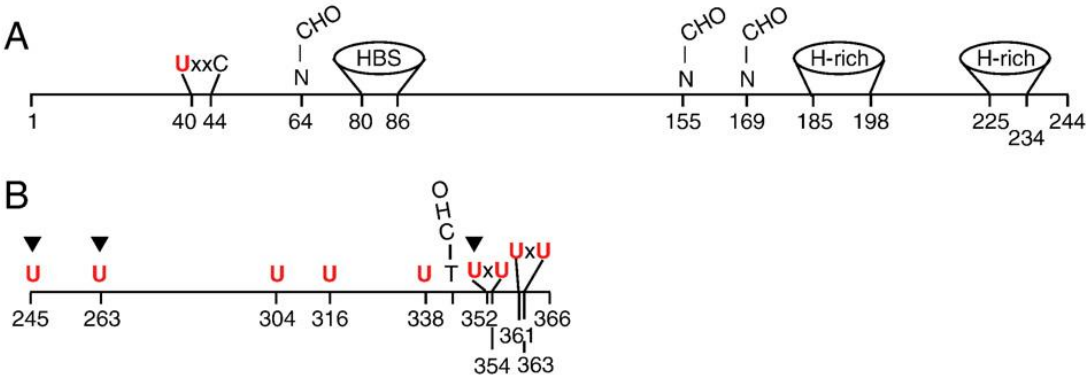


Figure 5: N and C terminal domains for SEPP1.SEPP1's ten Sec residues are depicted by the red 'U' codon. Within the N-terminal domain, one Sec molecule is present with a thioredoxin-like motif (UxxC), two histidine rich motifs (H-rich) are accountable for the heparin binding properties of the protein, the heparin binding site (HBS) allows heparin binding in a pH sensitive manner and there are three carbohydrates within the N-domain (N-CHO). The remaining 9 Sec molecules are contained within the final third of the protein (C-terminal). (A) N-terminal (B) C-terminal. Figure adopted from (Burk and Hill, 2009).

1.1.5.3 Iodothyronine Deiodinases

The family of three Sec containing DIO (iodothyronine deiodinase 1 (DIO1), iodothyronine deiodinase 2 (DIO2) and iodothyronine deiodinase (DIO3)) are vital selenoproteins implicated in correct thyroid hormone function (Larsen and Zavacki, 2013). All three act as oxidoreductases, with an active site containing one Sec residue that is responsible for catalysing the activation or deactivation of 3,5-diiodo-L-thyronine (T2), T3, 3,3',5'-triiodothyronine (reverse triiodothyronine, rT3) and T4 (Roman et al., 2014). All three membrane proteins are similar in structure, differing in their cellular localisations. DIO1 and DIO3 are located within plasma membranes, whereas DIO2 is located within the endoplasmic reticulum (Larsen and Zavacki, 2013).

The DIO's are instrumental in maintaining and controlling thyroid hormone homeostasis (Labunskyy et al., 2014). DIO1 and DIO2 catalyse outer ring deiodination of T4 to produce active T3 (Labunskyy et al., 2014). DIO1 is responsible for 80% of the total circulating T3 and is found primarily in the liver, thyroid and kidney whilst DIO2 is found to be highly active in the central nervous system, anterior pituitary and brown adipose tissue (Chan et al., 2003). In comparison, DIO3 (and under certain conditions, DIO1) are responsible for the deactivation of T4 and T3 into rT3 and T2 by catalysing the deiodination of the inner ring (Figure 6). During pregnancy, DIO3 prevents against excessive fluctuations in foetal thyroid hormone supply and is present in abundance in the placenta and uterus (Larsen and Zavacki, 2013).

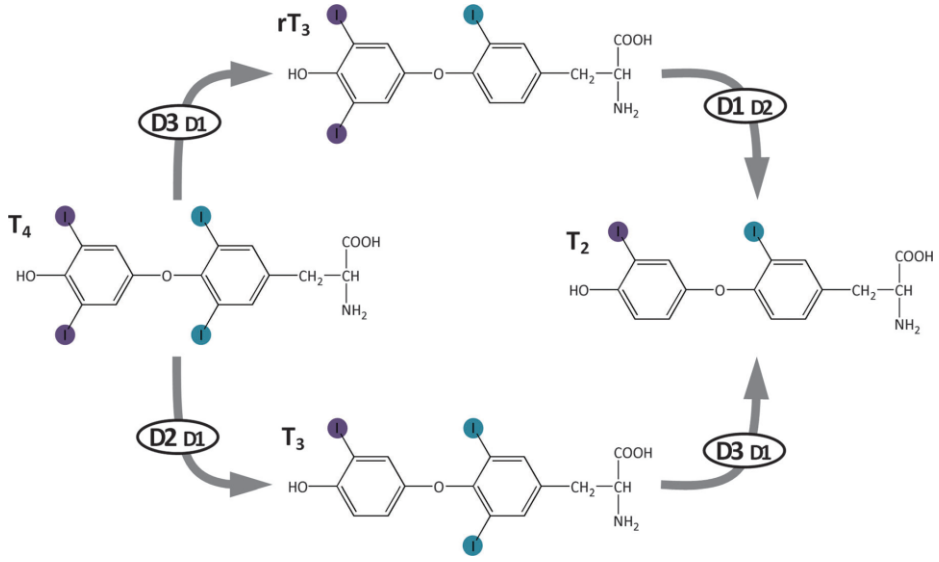


Figure 6: DIO regulation. The diagram depicts the metabolism of the thyroid hormones mediated by DIO1, DIO2 and DIO3. Figure adopted from Roman et al. (2014)

1.1.6 Selenoprotein Hierarchy

The adverse consequences of selenium deficiency result in the premature termination of proteins at the 'UGA' codon and the increased degradation of selenoprotein mRNA; both leading to decreased selenoprotein expression (Wingler et al., 1999). Therefore, in order to manage protein expression in selenium deficient states, selenoproteins are ranked in a selected hierarchy dependant on their biological importance (Wingler et al., 1999; Burk and Hill, 2015). The hierarchical principles controlling selenoprotein expression aim to distribute selenium supply to the most vital selenoproteins during periods of deficiency. Subsequently, this allows vital functions such as metabolism and growth to continue unimpaired (Schomburg, 2011). Two main principles are witnessed in higher mammals: 1) the biosynthesis of preferential selenoproteins and 2) unequal tissue distribution of selenium (Schomburg and Schweizer, 2009). The expression of selenoproteins are directly affected by selenium bioavailability. Selenoproteins such as GPx1, GPx3 and selenoprotein W are ranked low in the selenoprotein hierarchy resulting in downregulation in periods of selenium deficiency. This is in contrast to proteins such as TrxR1, TrxR3, SEPP1 and DIO who rank highly and are less dependent on dietary selenium intake (Labunskyy et al., 2014; Brigelius-Flohe and Maiorino, 2013; Solovyev et al., 2018). A number of studies in rodents have explored this principle. Bermano et al. (1995) investigated the hierarchical system of three selenoproteins; DIO1, GPx1 and GPx4. Results indicated that the dietary intake of selenium regulated the activity of selenoprotein expression and the corresponding mRNA concentrations. GPx1 concentrations fell rapidly in a selenium deficient state, whereas GPx4 and DIO1 concentrations were continually maintained. Hill et al. (1992) also found similar results. Under selenium deficient conditions, decreases in liver SEPP1 and GPx1 concentrations were measured, however GPx1 fell rapidly before any decreases in SEPP1 were witnessed. A pre-translational effect was also noticed in the selenoprotein mRNA. DIO1 and SEPP1 mRNA was found to be more resistant to selenium deficiency compared to GPx1. It was hypothesised that the limited selenocysteyl-tRNA$^{[Ser]Sec}$ machinery was being directed towards the synthesis of more important selenoproteins.

Specific organs also have the increased capacity to retain selenium as per its individual requirements. The brain, thyroid and reproductive organs store selenium more efficiently compared to the liver, kidney and muscle (Berry, 2005). This was shown in a study conducted by Behne et al. (1988) who found that selenium distribution in rats were directed to certain tissues in periods of selenium insufficiency. Retention of selenium was found to be 20-50 times higher in organs such as the brain, ovaries and endocrine glands compared to the kidneys, liver and heart.

1.1.7 Selenium Requirements

Selenoproteins are dependent on sufficient selenium intake in order to guarantee optimal protein expression and avoid impaired functionality (Mostert, 2000). Due to the large geographical variation in selenium concentration (as described in section 1.1.2), there are no internationally recognised reference ranges for serum selenium concentrations (Thomson et al., 2007b). Therefore, dietary values of selenium intake are based on maintaining a serum selenium concentration that will sustain and maximise optimal GPx output (Ashton et al., 2009). This threshold varies between studies, however levels are commonly expressed as being between 90-100 µg/L (Stoffaneller and Morse, 2015; Combs, 2001; Thomson et al., 1977). Recommended dietary selenium intake also varies by country. Within the UK and Belgium, Dietary Reference Values (DRV) for selenium intake is 60 µg/day for adult women, increasing to 75 µg/day for adult men and lactating women. The UK does not suggest increasing selenium intake during pregnancy (Public Health England, 2016b), however Belgium recommend an increase of 5 µg/day (Mehdi et al., 2013). This is in contrast with the United States of America (USA) and the WHO recommendations where Recommended Dietary Allowances (RDA) measure 55 µg/day for both sexes in the USA (Institute of Medicine Food and Nutrition Board (US), 2000) and 40 µg/day for males and 30 µg/day for females as per the WHO guidelines (Ventura et al., 2017). The changes in RDAs/DRVs between countries can be attributed to the varying geochemical conditions witnessed between differing areas of the world (see section 1.1.2).

Some countries have taken the step to supplement selenium into mineral fertilisers to increase selenium intake. For example, since the 1980's, Finland have fortified crops and animal feed with sodium selenate due to a lack of bioavailable selenium in its soils. As a result, selenium intake and serum concentrations within the population has risen significantly over the past 40 years. Dietary intake has increased from 0.025 mg/day to 0.8 mg/day; this intake currently meets requirements as set by the European Union (EU) and USA regulatory bodies. Average serum selenium concentrations have also risen significantly in this time. Before selenium supplementation, the mean concentration was 0.89 µmol/L; in the 2010's the measurement stood at 1.4 µmol/L, making it above serum selenium levels seen in most of Europe (Alfthan et al., 2015).

There is a fine line between too little and too much selenium in the diet. Chronic toxicity can lead to selenosis. Manifestations of this are signalled by brittle/loss of hair, gastrointestinal problems and foul breath. To prevent excessive intake, many countries such as the USA and the WHO have set upper limits of 400 µg/day and 300 µg/d respectively (Mistry et al., 2012).

1.1.8 Selenium and Health

Two main disease states as a result of low selenium intake are 'Keshan' disease and 'Kaschin-Beck' disease. Prevalence of these diseases are common in mountainous areas of rural China and Siberia (Navarro-Alarcon and Cabrera-Vique, 2008). Keshan disease was first discovered in the 'Keshan' area of China, displaying as an endemic juvenile cardiomyopathy occurring in 2-10 year olds and in some instances, women of child bearing age (Combs, 2001). Kaschin-Beck disease, also referred to as "big-joint disease" (Hartikainen, 2005), is a rheumatoid condition commonly seen in 5-13 year olds. It is caused by oxidative damage to cartilage resulting in deformed bone structure i.e. shortened fingers and toes, enlarged joints and in very extreme cases, dwarfism (Navarro-Alarcon and Cabrera-Vique, 2008). Disease states were distributed along a belt of land running from the northeast to the southwest of the country known to contain soils with very low selenium bioavailability (Hartikainen, 2005).

Adverse cardiovascular events in individuals have been linked with low selenium status. A study conducted in Finland before mandatory selenium fertilisation assessed the incidence of coronary heart disease (CHD), myocardial infarction (MI) and their associations with selenium status. Results found that serum selenium below 45 µg/L statistically increased the risk of both CHD and MI (Salonen et al., 1982). Oxidative stress has been shown to damage vascular endothelial cells, resulting in

the exacerbation of cardiovascular disease (CVD). Since certain selenoproteins are renowned for their oxidative defence mechanisms, it has been hypothesised that adequate selenium intake could prevent the onset of CVD and defend against reactive oxidative species (ROS) (Roman et al., 2014). Low levels of selenium have also been linked with male infertility. GPx4 and SEPP1 are two selenoproteins involved in spermatogenesis. GPx4 found in the mitochondrial capsule is responsible for two key processes (Mistry et al., 2012). During early phase spermatogenesis, GPx4 antioxidative properties prevent against oxidative stress. For normal spermatozoa formation during later phase spermatogenesis, GPx4 is crucial for creating crosslinks with proteins, becoming a structural component within the flagellum which aids sperm motility. A study in Japan showed that a proportion of infertile men had GPx4-defective sperm (Rayman, 2012). SEPP1 is also required in the testis to facilitate selenium transport, as shown in SEPP1 knockout mice. SEPP1 transport to the apolipoprotein E receptor-2 was insufficient, leading to impaired spermatogenesis and defective spermatozoa, resulting in infertility (Burk and Hill, 2009). Sufficient dietary selenium has been shown to stimulate and enhance immune response by improving the proliferation of activated T-lymphocytes, increasing natural killer cell activity and increasing cytotoxic lymphocyte-mediated tumour cytotoxicity (Ventura et al., 2017; Rayman, 2012). In contrast, adverse immunocompetence such as low T-lymphocyte counts and lymphocyte proliferation have both been associated with low selenium concentration (Rayman, 2000; Brown and Arthur, 2007).

Proving a conclusive link between selenium concentration and cancer still remains challenging (Roman et al., 2014). A landmark trial performed by Clark et al. (1996) was one of the first to link selenium concentration and incidences of carcinoma. Whilst there was no association between selenium and the primary outcome of the study, cancer mortality and the frequency of site specific cancers such as prostate, lung and colorectal was significantly lower for participants receiving 200 µg selenium supplementation, compared to those in the placebo group. However, reanalysis of this study data by Reid et al. (2002) concluded that lung cancer incidence was not significantly associated with selenium supplementation. Further research is required to determine definitive links between selenium, cancer and the mechanisms involved.

1.1.8.1 Selenium Deficiency and Pregnancy

During pregnancy, adequate placental transfer of selenium is required for foetal selenoprotein production and thyroid hormone metabolism to enable normal growth and development (Zimmermann and Kohrle, 2002). Impaired transfer due to selenium insufficiency has been linked to adverse birth outcomes (Burk et al., 2013), however, few studies so far have investigated these effects. Studies that have investigated this potential link found low levels of selenium and selenoproteins during pregnancy were associated with conditions such as pre-eclampsia, pre-term birth and low birth weight.

A Bangladeshi study conducted by Haque et al. (2016) investigated the effects of selenium concentration and pre-eclampsia. In 192 pregnant women, mean selenium concentration was shown to be significantly lower in the pre-eclamptic group compared to control groups (23.76 µg/L and 32.18 µg/L respectively, $p<0.05$). Mistry et al. (2008) found similar results in a cohort of UK pregnant women. Mean selenium concentration in women who had pre-eclamptic pregnancies was significantly lower in comparison to mothers who did not (39.7 µg/L and 58.4 µg/L respectively, $p<0.001$). A further study performed by Rayman et al. (2003) found that toenail selenium levels in moderately/severe pre-eclamptic pregnant women were significantly lower than pregnant women without the condition. In addition, when selenium concentration was ranked into thirds, it was found that half the pre-eclamptic women were classified in the lowest third. A Dutch study performed by Rayman et al. (2011) investigated selenium status and the association with pre-term birth. Logistic regression showed that women with a selenium concentration in the lowest quartile had twice the risk of pre-term birth (odds ratio: 2.0, 95% confidence interval (CI): 1.19-3.47). Low selenium during pregnancy has also been associated with low birthweight. A study conducted in the USA by Bogden et al. (2006) found that mothers with a serum selenium concentration in the lowest ten percent during early pregnancy (median 8.7µg/L) were significantly associated with low birth weight. These studies highlight the potential importance of adequate selenium concentration in pregnancy to prevent adverse birth outcomes.

1.2 Iodine

1.2.1 Iodine Geochemistry

Iodine concentrations are known to be unevenly distributed within the environment (Zimmermann et al., 2008). Levels are heavily dependent on a number of factors including geographical location, rock type, iodine fixation potential of the soil, climate, topography and the proximity to the sea (Johnson, 2003). Soil geochemistry provides the link between the environmental iodine and the food chain. As iodine concentrations are reliant solely upon dietary intake, the specific geochemistry of an area is incredibly important for iodine adequacy (Johnson, 2003). Mountainous areas such as the Himalayas and the French Alps are susceptible to a lack of iodine due to igneous rock having a lower iodine content in comparison to sedimentary rock (Johnson, 2003). The 'goitre belt' prevalent in the UK during the early to mid-1900's (Figure 7) lay on carboniferous limestone rock which was thought to cause insufficient iodine levels in large swathes of the country (Saikat et al., 2005). Incidences of goitre (enlarged thyroid) were reported from Cornwall, through the Midlands and up into areas of Derbyshire and was commonly referred to as the 'Derbyshire Neck' (Bath and Rayman, 2015).

Figure 7: UK goitre belt. The shaded area of the UK indicates the widespread prevalence of goitre in past decades. Adopted from Phillips (1997).

1.2.2 Dietary Sources of Iodine

In the UK, seafood, fish, eggs and dairy account for the majority of iodine intake as shown in Table 3 (SACN, 2014). Dairy consumption is the main source of dietary iodine across all UK population groups (Public Health England, 2018) with 34% of all iodine intake for adults (aged 19-64) coming from dairy based produce. In the farming industry, fortified livestock feed and iodine containing cleaning products have been shown to increase iodine levels in meat and dairy produce (Zimmermann, 2009a). In the 1930's, the UK saw a steady rise in milk iodine levels as farmers implemented iodised cattle feed along with iodised salt licks as they were thought to have major reproductive benefits for the livestock. This coincided with an increase in milk consumption, which in turn saw iodine levels rise within the UK population (Phillips, 1997). Conversely, a number of edible plants and vegetables are thought to inhibit the iodine uptake process due to competition from glucosinolates. For example, broccoli, kale, turnips and cabbage (Zimmermann, 2009a). The first report of this was in 1928 when cabbage feeding in rats resulting in goitre development (Chesney et al., 1928). Soy bean has also been identified as a goitrogen due to its role in the impairment of thyroid peroxidase activity (Eastman and Zimmermann, 2018).

Food	Iodine content (µg/100g)
Mussels, cooked	247
Cod, baked	161
Egg yolk, boiled	137
Eggs, whole, boiled	52
Milk chocolate	51
Sea salt	50
Whole milk, pasteurised, average	31
Semi-skimmed milk, pasteurised, average	30
Skimmed milk, pasteurised, average	30
Cheddar cheese	30

Table 3: Iodine rich food sources in the UK. The table displays iodine rich foods within the UK and the estimated iodine content (µg/100g). Adapted from SACN (2014).

1.2.3 The Thyroid Gland

The thyroid is a butterfly-shaped endocrine gland responsible for the production of the two key iodine containing hormones; T3 and T4. It is located in the lower part of the neck, spanning either side of the trachea consisting of a right and left lobe – both are connected by tissue called the isthmus (Figure 8) (Martin;, 2015). Each lobe measures approximately 2 cm in thickness, 4 cm in length and has a combined weight of around 15-25 g, making it one of the largest endocrine glands in the body (based on a normal, disease free state) (Mohebati and Shaha, 2012). At a microscopic level, the epithelial cells (thyrocytes) are arranged into a follicle around a protein rich matrix called the colloid (see top left Figure 9). The follicular cells are orientated to facilitate the transportation of iodine into the cell from the capillaries through the basolateral membrane (Nussey and Whitehead, 2001), along with the transfer of precursors in and out of the follicular lumen at the apical surface (Di Jeso and Arvan, 2015).

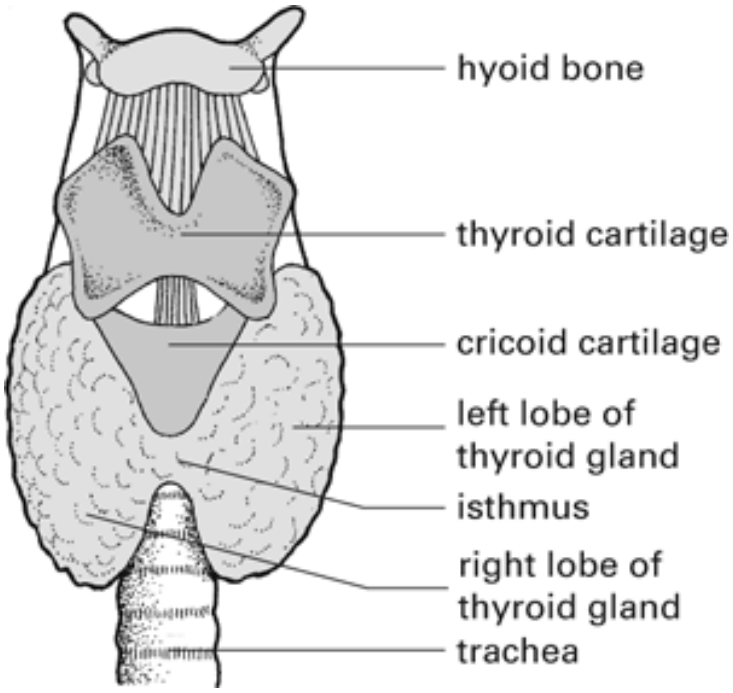


Figure 8: The thyroid gland. The diagram shows the key parts of the thyroid gland and its location within the neck. Adopted from Martin; (2015).

1.2.4 Iodine Metabolism

Iodine metabolism is depicted in Figure 9. The first stage of iodine metabolism is known as iodine trapping. After ingestion, iodine is converted to iodide, absorbed through the small intestine and transported through the plasma to the thyroid gland (World Health Organisation, 2004). Iodide is then taken up into the thyrocytes by the sodium/iodide symporter found at the basolateral membrane of the follicular cell. One iodide ion and two sodium ions are transported into the cell against both chemical and electrical gradients with sodium returning to the capillary via the sodium/potassium ATPase. Simultaneously, thyroglobulin is produced at the rough endoplasmic reticulum in the thyrocytes, where individual tyrosine molecules combine to form a complete peptide (140 tyrosine molecules per thyroglobulin molecule). Fully assembled, the vesicle containing thyroglobulin moves via the Golgi apparatus to the apical membrane where it enters into the follicular colloid via exocytosis. Newly imported iodide molecules move through the follicular cell to the apical membrane where they are transported via the pendrin transporter into the colloid space. Iodide is then oxidised, with two iodide ions required to produce one diatomic iodine molecule. This oxidation is catalysed by both thyroid peroxidase (TPO) and H_2O_2. The resulting iodine molecule covalently links with the tyrosyl residues of thyroglobulin to produce either monoiodotyrosine (MIT) or diiodotyrosine (DIT) precursors. Iodination is followed by a coupling reaction of the tyrosyl molecules where either T3 or T4 is produced (two DIT molecules couple to form T4, one MIT and one DIT molecule couple to form T3). Under the influence of thyroid stimulating hormone (TSH), iodinated thyroglobulin then moves back into the cell via endocytosis (Nussey and Whitehead, 2001). Once in the cell, lysosomes fuse with the colloid droplets, cleaving T3, T4, MIT and DIT from the thyroglobulin molecule in the cytoplasm. Around 10% of the newly formed T4 is mono-deionised to T3 before both hormones move towards the basolateral membrane. Remaining MIT and DIT molecules are completely deiodinated and recycled by tyrosine dehalogenase. The new thyroid hormones are secreted into the bloodstream where they circulate in either bound or unbound forms (Nussey and Whitehead, 2001; Ahad and Ganie, 2010; Di Jeso and Arvan, 2015; Rousset et al., 2015).

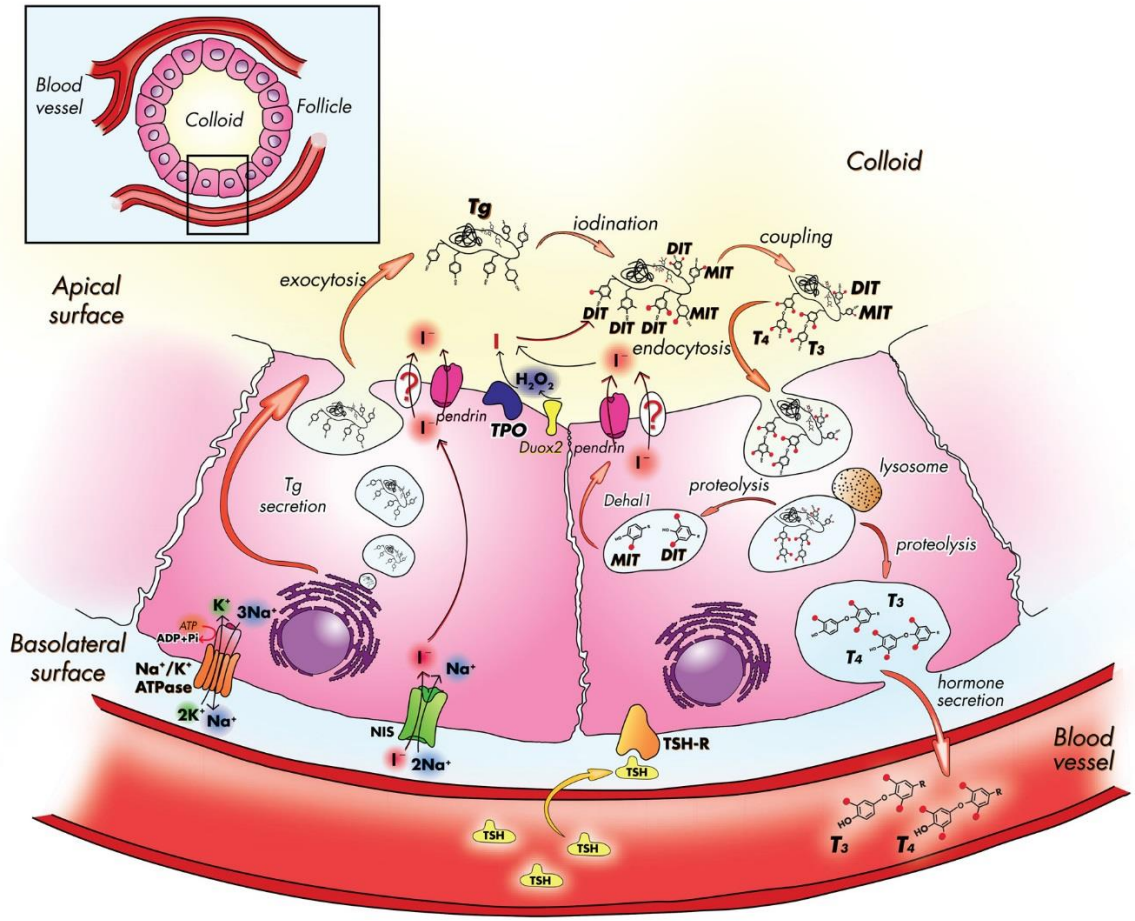


Figure 9: Iodine metabolism within the thyrocytes. Depiction of iodine transportation and synthesis within the thyroid follicular cell to produce T3 and T4 hormones. Adopted from Di Jeso and Arvan (2015).

1.2.5 Key Thyroid Hormones

1.2.5.1 Thyroxine and Triiodothyronine

As described in section 1.2.4, T3 and T4 are metabolised in the follicular cells of the thyroid gland and excreted into the blood stream. Approximately 100 µg of thyroid hormones are released from the thyroid daily (Nussey and Whitehead, 2001), with the T4 prohormone excreted at nearly 10-fold the concentration of active T3 (Larsen and Zavacki, 2013). The majority of T4 and T3 is then transported through the circulatory system bound to carrier proteins, such as thyroxine binding globulin (TBG) and albumin. Only 0.03% of T4 and 0.3% T3 travel unbound as free 3,5,3',5'-tetraiodothyronine (fT4) and free 3,5,3'-triiodothyronine (fT3) (Refetoff S., 2015). The bound fraction acts as a 'circulating reservoir' with only the unbound fraction of the thyroid hormones acting upon target cells (Nussey and Whitehead, 2001).

T4 is converted by selenium containing DIOs into either active fT3 or non-active rT3. fT3 binds to high affinity T3 nuclear receptors in the nucleus of the cell (World Health Organisation, 2004). This helps to regulate downstream gene expression which in turn effects a plethora of bodily processes such as somatic growth and central nervous system development in children, along with regulation of the metabolic rate and the synthesis of key enzymes in adults (Velasco et al., 2018).

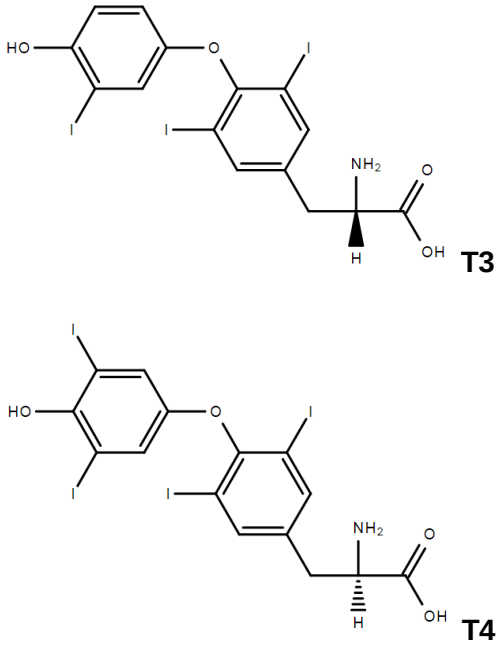


Figure 10: T3 and T4 chemical structures. The iodine positions for T3 and T4 are depicted by Figure 10. 64% of the molecular weight of T4 is composed of iodine compared to just 58% for T3. Figure adopted from Royal Society of Chemistry (2015)

1.2.5.2 Thyroid Stimulating Hormone

TSH is produced by the anterior pituitary gland at the base of the brain. It is a large glycoprotein weighing 28 KDa and consisting of two subunits – α and β. TSH is responsible for regulating thyroid hormone homeostasis via a negative feedback mechanism controlled by the hypothalamic-pituitary (HPT) axis (Nussey and Whitehead, 2001). As the brain specific DIO2 rapidly converts T4 into T3, low levels of T4 are recognised and thyrotropin releasing hormone (TRH) is released from the hypothalamus into the circulatory system (Larsen and Zavacki, 2013). TRH stimulates thyrotrophs in the anterior pituitary to upregulate TSH production which allows an increased concentration of iodine into the cell via the sodium/iodide symporter. This results in increased hormone synthesis and the release of T4 and T3 (Pirahanchi and Jialal, 2018). In contrast, when T4 and T3 levels are excessive, TSH production ceases and the hypothalamus secretes somatostatin, inhibiting TSH release and in turn, reducing intestinal iodine uptake and renal retention (SACN, 2014).

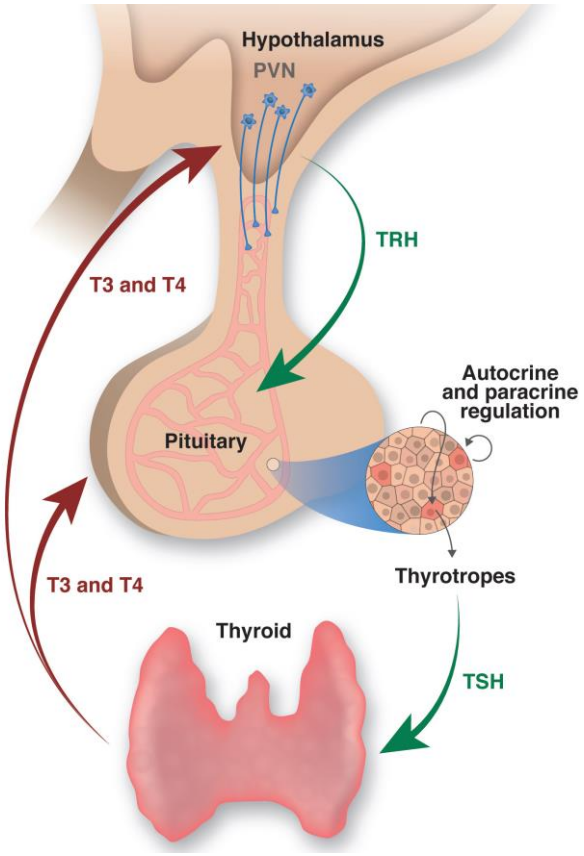


Figure 11: The HPT axis. A depiction of the negative feedback mechanism controlled by the HPT axis. Low T3 and T4 concentrations stimulate TRH release, resulting in the upregulation of TSH in the pituitary gland. This stimulates the thyrocytes to increase thyroid hormone production. Figure adopted from Ortiga-Carvalho et al. (2016).

1.2.5.3 Thyroglobulin

Thyroglobulin is one of the largest molecules within the human proteome. Located on chromosome 8, the dimeric 660 KDa protein is exclusively synthesised in the thyroid gland making it the most specific and abundant intrathyroidal protein in the human body (Zimmermann and Andersson, 2012). Stored within the colloid, its concentrations range from ~200-300 g/L (Rousset et al., 2015), where it facilitates the production of both T3 and T4 by the covalent linkage of iodide to tyrosyl residues to produce MIT and DIT (described in section 1.2.4). About 40% of T4 is synthesised at the extreme N-terminus of the thyroglobulin protein (tyrosyl 5), compared to roughly 20% at tyrosyl site 2554. T3 is predominantly formed at the C-terminal (Rousset et al., 2015) On average, only a fraction of the tyrosine molecules are actually iodinated (10-20 tyrosyl molecules per thyroglobulin molecule out of a possible 140) (Di Jeso and Arvan, 2015).

As thyroglobulin is thyroid specific, it is a good marker of thyroid status. Thyroglobulin containing endosomes can cause transcytosis where small amounts are released into the circulation (Zimmermann et al., 2013). During iodine deficiency, an increase in TSH causes the follicular cells of the thyroid to enlarge causing increased amounts of thyroglobulin to be released into the blood stream. For example, in a normal, iodine sufficient individual, thyroglobulin concentrations can range from 5-14 µg/L compared to that of an individual with goitre displaying mean concentrations of 94-208 µg/L (Ma and Skeaff, 2014).

1.2.6 Iodine Requirements

Iodine requirements are dependent upon population demographic. Regulatory bodies such as the WHO and the United States Institute of Medicine (IoM) differ on their recommendations as seen in Table 4 (World Health Organisation, 2007; Zimmermann et al., 2008). UK specific iodine requirement values were set by the Committee on Medical Aspects of Food and Nutrition Policy (COMA) in 1991. To this day, Reference Nutrient Intakes (RNI) remain unchanged. Both adult men and women are required to have an iodine intake of 140 µg/day, 10 µg less than recommendations provided by the WHO and IoM (COMA, 1991).

Population	WHO (µg/day) [RNI]	Population	IoM (µg/day) [RDA/AI]	Population	COMA (µg/day) [RNI]
Infants (0-5 years)	90	Infants (0-12 months)	110-130	Infants (0-3 years)	50-70
Children (6-12 years)	120	Children (1-8 years)	90	Children (4-14 years)	100-130
		Children (9-13 years)	120		
Adolescent/Adult (+12 years)	150	Adults (+14 year)	150	Adolescent/Adult (+15 years)	140
Pregnancy	250	Pregnancy	220	Pregnancy	N/A
Lactating Women	250	Lactation	290	Lactation	N/A

Table 4: Recommendations for dietary intake of iodine. Daily iodine requirements (µg/day) in different population groups according to the WHO, IoM and COMA. Adapted from Zimmermann (2009a) and COMA (1991). RDA: Recommended Dietary Allowance; AI: Adequate Intake; RNI: Recommended Nutritional Intake.

1.2.6.1 Iodine Requirements during Pregnancy

Pregnancy is associated with major changes in thyroid function and iodine requirements (Glinoer, 2007). This is the result of three main factors: maintaining normal maternal thyroid gland function (euthyroidism) whilst also transferring T4 to the foetus in early gestation for growth and development; transferring iodine across the placenta for foetal thyroid hormone production in later gestation and to compensate for the increase in renal iodine clearance (Zhang et al., 2017).

In early pregnancy, an increase in TBG stimulated by a rise in oestrogen and enhanced hepatic synthesis starts to effect hormone homeostasis (Elnagar et al., 1998; Lazarus, 2011). Simultaneously, increased renal blood flow and glomerular filtration rate during early gestation leads to an increase in renal iodine clearance by as much as 50%. As a result, the circulating plasma iodine pool decreases significantly (Glinoer, 2007). Towards the end of the first trimester, the concentrations of human chorionic gonadotrophin (hCG) has a weak, stimulatory effect on thyroid hormone production (Glinoer, 2004), resulting in lower TSH values and higher concentrations of free thyroid hormones (Fantz et al., 1999). Daily maternal T4 increases of approximately 50% require an additional ~75-150 µg of T4 to maintain both placental iodine transfer and maternal euthyroidism. This translates to an extra 50-100 µg of dietary iodine intake per day (Delange, 2007). As the foetal thyroid gland does not function until roughly 12 weeks gestation, it is imperative that the mother has sufficient iodine to keep up with the demands throughout pregnancy to sustain euthyroidism and ensure normal brain development in utero for the foetus. (Glinoer, 2007). The foetal thyroid starts to concentrate iodine in preparation for foetal pituitary TSH stimulation at approximately 20 weeks of gestation (Leung et al., 2011). During the second trimester, maternal iodine crosses to the placental unit allowing the foetal thyroid gland to produce thyroid hormones independently from the mother (Glinoer, 2004).

Due to these factors, dietary iodine requirements throughout pregnancy and lactation are higher than in non-pregnant, non-lactating adults (Delange, 2007). As displayed in Table 4, the WHO recommend approximately 10% more iodine per day for pregnant women (250 µg/day) compared to guidance set by the IoM (220 µg/day). There are currently no specific iodine recommendations for UK mothers during pregnancy and lactation (COMA, 1991).

1.2.7 Iodine Assessments

Two common assessments used to determine iodine status are urinary iodine concentration (UIC) and biomarker analysis (serum T4, TSH and thyroglobulin) (Zimmermann, 2009a). UIC determination is a good indicator of recent iodine status as approximately 90% of all dietary iodine intake is excreted from the body via urine. As a non-invasive procedure, urine samples are easy to obtain, with spot urines being the most commonly used due to their improved practicality over 24 hour urine collections (Zimmermann, 2012). There are several methods to assess UIC including colorimetric assays and inductively coupled plasma mass spectrometry (ICP-MS). Epidemiological criteria for UIC as set by the WHO is displayed in Table 5 (World Health Organisation, 2007). Both serum TSH and serum thyroglobulin are also used as markers of iodine status. TSH increases in response to a low circulating level of T4. TSH testing is effective in neonates, however less sensitive in children and adults. As thyroglobulin is a specific thyroidal protein, its assessment is a better indicator of iodine status in children and adults with an increase in blood thyroglobulin signalling a potential iodine deficiency (Zimmermann, 2009a).

UIC (µg/L)	Iodine Intake	Iodine Status
School Aged Children		
<20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Adequate iodine deficiency
200-299	More than adequate	More than adequate
>300	Excessive	High risk
Pregnant Women		
<150	Insufficient	-
150-249	Adequate	-
250-499	More than Adequate	-
>500	Excessive	-
Lactating Women		
<100	Insufficient	-
>100	Adequate	-
Children less than 2 years of age:		
<100	Insufficient	-
>100	Adequate	-

Table 5: The WHO UIC criteria. UIC criteria used to assess iodine nutrition in differing population groups. Criteria is based on median UIC status in school aged children. Adopted from World Health Organisation (2007).

1.2.8 Iodine Deficiency

Iodine Deficiency Disorders (IDD) are the manifestation of adverse clinical effects resulting from insufficient iodine intake and inadequate thyroid hormone production (Zimmermann, 2012). IDD are the “most common cause of preventable mental retardation and underdevelopment in the world” as defined by the WHO (World Health Organisation, 2007). The onset of IDD can vary depending on age as seen in Table 6. Pregnant and lactating women along with those under three years of age are important populations to monitor as iodine deficiency can be detrimental to long-term growth and development (World Health Organisation, 2004).

Foetus	Miscarriage Stillbirths Congenital anomalies Increased perinatal morbidity and mortality Endemic cretinism
Neonate	Neonatal goitre Neonatal hypothyroidism Endemic neurocognitive impairment Increased susceptibility of the thyroid gland to nuclear radiation
Child and adolescent	Goitre (Subclinical) hypothyroidism Impaired mental function Retarded physical development Increased susceptibility of the thyroid gland to nuclear radiation
Adult	Goitre with its complications Hypothyroidism Impaired mental function Spontaneous hyperthyroidism in the elderly Iodine induced hyperthyroidism Increased susceptibility of the thyroid gland to nuclear radiation

Table 6: Common IDD. Table 6 lists some of the common IDD seen in differing population groups. Table adopted from World Health Organisation (2007).

1.2.8.1 Iodine Deficiency during Pregnancy

Iodine deficiency during pregnancy has been linked with goitre (Alexander et al., 2017), congenital abnormalities, (Miller et al., 2016), endemic cretinism, spontaneous abortion and still birth (Arthur et al., 1999). Severe iodine deficient states give rise to low levels of circulating T4, causing the pituitary gland to stimulate production and secretion of TSH. The consequence of this continual, long-term TSH stimulation and low T4 results in the hyperplasia of the thyroidal follicular cells creating an enlarged maternal and foetal thyroid (goitre) (Ahad and Ganie, 2010; Alexander et al., 2017). With thyroid hormone receptors present in the foetal brain at 9 weeks, sufficient thyroid hormone concentrations are required for early myelination, normal neuronal migration and maturation of the foetal brain (Zimmermann, 2011). However, in iodine deficient states, insufficient thyroid hormone concentrations can cause severe brain damage and irreversible neurological abnormalities. This has the risk of manifesting as endemic cretinism in the offspring (Zimmermann, 2016). There are two forms of cretinism –neurological and myxoedematous. Neurological cretinism is characterised by deaf-mutism, motor spasticity of the limbs and mental retardation caused by impaired brain development (Ahad and Ganie, 2010). Myxoedematous is discussed in section 1.3. Whilst severe iodine deficiency in pregnancy has been well characterised, the effects of mild to moderate iodine deficiency are less conclusive (Hynes et al., 2013).

1.2.9 Preventing Iodine Deficiency

Iodination of dietary salt has been the main approach in combatting global iodine deficiency. Since 1994, the WHO, United Nations Children Fund (UNICEF) and the International Council for Control of Iodine Deficiency Disorders (ICCIDD) have recommended universal salt iodisation (USI) programmes be implemented in countries known to have populations at risk of severe and mild/moderate iodine deficiency (World Health Organisation, 2007). As of 2013, iodine fortification programmes were mandatory in 13 European countries, whilst 22 countries had yet to implement any legislation, including the UK (Lazarus, 2014). In countries that have implemented iodine fortification programmes, iodised salt is the main source of iodine, either from foods produced in mass industry or from household usage (Zimmermann and Andersson, 2012). For example, fortification of bread with iodised salt has improved iodine status within the population of Denmark (Miller et al., 2016).

1.3 Iodine, Selenium and Thyroidal Interaction

The thyroid contains the highest concentrations of iodine and selenium in the entire body (Wu et al., 2015). With the biological role of iodine only known to be implicated in thyroid hormone production, the interactions between the two micronutrients are pivotal for the synthesis and subsequent utilisation of thyroid hormone metabolism (Schomburg and Kohrle, 2008; Arthur et al., 1999). Two specific selenoprotein families have an influential effect on the thyroid hormone axis, GPx and DIO (Zimmermann and Kohrle, 2002).

Essential for thyroid hormone metabolism (see section 1.1.5.3) (Ventura et al., 2017), DIO are implicated in the activation and deactivation of thyroid hormones (Arthur et al., 1999). Inadequate selenium intake witnessed in the study of rats first suggested that high T4 and low T3 concentrations was the result of inadequate DIO expression, resulting in the impaired conversion of T4 to T3 (Arthur et al., 1999). Selenium and iodine deficiency can therefore lead to abnormalities in thyroid hormone synthesis and limited hormone activation due to decreased DIO activity, thereby impacting negatively on growth and maturation (Arthur et al., 1999). Under selenium deficient states, the decrease in DIO1 specifically stimulates the HPT axis to increase TSH concentration as it has been shown that TSH significantly increases DIO1 activity (Beech et al., 1995). However, an increased stimulation of TSH in selenium and iodine deficient states can also affect the normal architecture of thyroid gland due to impaired GPx activity resulting from insufficient selenium. GPx3 is an abundant selenoprotein within the thyrocytes and is responsible for the neutralisation of H_2O_2 (section 1.1.5.1). During hormone synthesis, the thyroid gland must produce enough H_2O_2 for iodination of the tyrosyl residues on the thyroglobulin protein. Any excess H_2O_2 concentrations are removed by GPx3 to prevent extensive oxidative damage to the components of the follicular cell i.e. DNA damage (Drutel et al., 2013). In iodine deficient states, T4 levels drop, hyper-stimulating TSH and the upregulation of thyroid hormone production. Consequently, this leads to an increase in H_2O_2. If whole body selenium is sufficient, high concentrations of GPx3 are present at the thyrocytes apical membrane to ensure the adequate defence of excess H_2O_2, preventing cell damage. However, in selenium deficient states (double iodine-selenium deficiency), a decrease in GPx3 means further oxidative damage from toxic lipid hydroperoxidases is likely to occur (Arthur et al., 1999).

The manifestation of severe iodine and selenium deficiency has led to disease states such as myxoedematous cretinism (Rayman, 2018). Myxoedematous cretinism was first linked to both iodine and selenium deficiencies following the results from a study based in Zaire, Central Africa (Diplock et al., 1990). Characterised by severe hypothyroidism, stunted growth, delayed sexual maturation and myxoedema (Schomburg, 2011), this form of cretinism is less severe than neurological cretinism onset (Drutel et al., 2013). Thiocyanate overload from non-detoxified cassava inhibiting iodide trapping within the placenta and foetal thyroid has also been linked to the onset of this disease (Eastman and Zimmermann, 2018).

Studies have confirmed that the severely impaired concentrations of both selenium and iodine directly effects thyroid hormone expression and activation. Wu et al. (2015) compared populations in two different counties of Shaanxi Province, China for thyroid disease based on their soil selenium status; the county of Ziyang had adequate soil selenium, Ningshan country had low soil selenium. The results showed that significantly less participants (18%) had thyroid disease in the adequate selenium county compared to that of the low selenium county (30.5%, $p<0.001$). In addition to this, participants in the lowest quintile (<47 µg/L selenium) had the highest prevalence of subclinical hyperthyroidism, overt hypothyroidism and enlarged thyroid, showing that the low selenium concentrations were probably insufficient for adequate selenoprotein expression needed for thyroid gland protection. A further study performed in 1992 in Zaire showed that after 50 µg selenium supplementation over two months, fT4 levels fell significantly, implying that increased DIO1 was accountable for these changes (Contempré et al., 1992).

Whilst studies have looked at selenium, iodine and thyroidal interactions within the general population, research between selenium and iodine during pregnancy has not yet been fully explored.

1.4 Study Aims

A number of studies have shown that the UK is mild-moderately deficient in iodine throughout pregnancy (Snart et al., 2019; Public Health England, 2018; Bath et al., 2015) Furthermore, the effects of iodine deficiency throughout gestation and its impacts on birth outcome have been extensively researched. With an important role in thyroid hormone metabolism and oxidative stress prevention, very limited research has assessed selenium status and its associated biomarkers throughout gestation. I hypothesise that within this study, selenium will decrease throughout pregnancy, selenium will be positively associated with both biomarkers and that selenium will have an impact on birth outcome.

Therefore to investigate this hypothesis, I aim to fulfil the following objectives:

1) Longitudinally quantify the levels of selenium, iodine and relevant biomarkers across pregnancy, giving an up to date assessment from a small cohort of women in the North of England.
2) Investigate the relationship between serum selenium and its biomarkers; SEPP1 and GPx3 during pregnancy.
3) Investigate the relationship between iodine and its biomarkers; fT3, fT4, TSH and thyroglobulin.
4) Investigate the relationship between serum selenium and iodine and the four thyroid hormones (fT3, fT4, TSH and thyroglobulin) during pregnancy.
5) Determine whether serum selenium is a reliable indicator of dietary selenium intake.
6) Investigate the impact of selenium status on birthweight.

The study has several novel aspects. It will be the first UK based project to observe the relationship between selenium and iodine and how these two micronutrients interact during pregnancy. Additionally, no previous study based within the UK has exclusively studied selenium concentration and its association with birthweight.

Methods and Materials

2.1 Participants and Sample Collection

2.1.1 Cohort Overview

Samples used in this study were obtained from the Health & Iodine Status in Babies (HIBA) longitudinal cohort (Health Research Authority Ethics Reference Number: 16/YH/0260; IRAS Ref: 204895 - Sheffield Research Ethics Committee, National Institute of Health Research). 70 mothers were recruited from August 2016 to November 2017 in Bradford, UK. Selenium, iodine and their respective biomarkers were monitored across pregnancy at 3 approximate time intervals; 13-15, 24- and 36-weeks' gestation (Figure 12). Women were approached at the sonography clinic at the Bradford Royal Infirmary where HIBA study information was given both verbally and in writing to those expressing an interest to enrol on the study (subject to meeting specific inclusion/exclusion criteria (Table 7)). Participants who met the criteria were invited back to a subsequent appointment at the Bradford Institute for Health and Research Clinical Research Facility where the study information was reiterated, participant queries answered and consent forms signed. Participants were made aware that they could withdraw at any point of the study. At the first visit (12/±3 weeks), research nurses administered an initial questionnaire (section 2.1.2), took anthropometric measurements such as weight and height, conducted a thyroid assessment (section 2.1.3), collected dietary assessments (section 2.1.4) and collected blood and urine samples (sections 2.1.5 and 2.1.6 respectively). The subsequent two visits were performed either in the clinic or at home as preferred by the participant. If the target week could not be met, visits were held either three weeks before the target week or three weeks after the target week. Meetings not possible within the intended timeframe were missed; participants would go on to attend subsequent visits. Mothers were reimbursed with a £10 voucher for each visit attended, in addition to any travel expenses.

All participant information was kept anonymous throughout the duration of the study. Identification was done by assigning each participant with a unique study number. Mother and baby records detailing information such as gestational age and birth weight was only accessed with prior consent. These records were used in the data analysis to assess for birth outcome (section 3.8)

2.1.1.1 Inclusion/Exclusion Criteria

Prior to participants commencing the study, each mother needed to meet specific inclusion and exclusion criteria as seen in Table 7.

Inclusion	Exclusion
Female	Prior history of thyroid disease
Confirmed as Pregnant at 9-15 weeks	Use of thyroid medication i.e. thyroxine
Aged 18-40	
Able to give informed consent	
Healthy with no known family history of thyroid conditions.	

Table 7: HIBA inclusion and exclusion criteria at recruitment.

2.1.2 Questionnaires

A comprehensive questionnaire was administered to all participants at the first visit by the recruitment team in Bradford (see Appendix 1: HIBA Questionnaire Examples). It assessed in detail the demographic information of all mothers including background, household information, education and circumstances. Questions also focussed on health such as prior medical history, their current pregnancy and their general wellbeing. All subsequent study visits included a brief follow-up questionnaire.

2.1.3 Anthropometric Assessments and Thyroid Inspection

Anthropometric assessments of weight and height were taken at each of the three visits. Participants were asked to remove any footwear prior to height assessments. Heavy articles of clothing were removed prior to weight assessments. Height was recorded with legs straight, heels together and head within the Frankfurt Horizontal Plane. Weight was recorded by participants standing in the middle of electronic scales. Thyroid inspections were also performed. In brief, a steady pulse and signs of tremor was initially measured. Participants eyes were assessed for protrusion and lid lag. Signs of goitre on the neck were measured by eye, during which participants were asked to swallow water to assess movement. Palpation techniques were further used if goitre was suspected.

2.1.4 Dietary Assessment

24-hour dietary assessments were taken at each visit using the Medical Research Council funded myfood24 dietary tool (University of Leeds, https://www.myfood24.org/web/). The extensive food database allows users to track, monitor and analyse the nutritional intake of individuals both accurately and easily. By selecting specific foods and the portion sizes, this database calculates nutritional data such as energy intake (kcal), fat (g), carbohydrates (g) and micro/macronutrients. This information can then be interpreted for further data analyses. Participants were asked to recall the food they had consumed within the previous 24 hours. Food intake was recorded chronologically by inputting into the system the brand name/exact food or drink; failing that either a similar item from a different brand was selected or the food item was replicated using a recipe builder. Portion size was assessed by asking participants how much they had consumed of each item based on photographic guides, grams or pack sizes. Higher portion sizes were manually entered. Lastly, participants were asked to recall any drinks consumed either with meals or throughout the day. Additional comments were added at the end of the dietary recall e.g. bouts of vomiting. Food data was inputted using a tablet device. If this was unavailable, the diary was recorded on paper and entered online retrospectively. Food diaries were labelled using a unique identifier in the format 'HIBA01234Visit ('01234' representing the participant identifier; 'Visit' representing the visit number).

2.1.5 Blood Collection and Processing

At each of the three visits, 30mL intravenous blood samples were collected. In brief, red top serum Vacuette© tubes (Greiner Bio-One Ltd, UK) were used to collect whole blood from participants. After collection, coded samples were inverted and left to clot for at least 30 minutes. Samples were centrifuged at 4°C for 10 minutes. The top layer of serum was collected and aliquoted in duplicate. After processing, samples were stored at -80°C at the Bradford Royal Infirmary until shipment on dry ice to the University of Leeds. Samples were kept at -80°C prior to analysis.

2.1.6 Urine Collection and Processing

Urine samples were collected at each of the three visits. In brief, mid-stream urine was collected by participants into collection tubes (Medline Scientific, UK). Collected samples were aliquoted in duplicate and centrifuged for 10 minutes at 4°C. Samples were barcoded and stored at -80°C at the Bradford Royal Infirmary until shipment on dry ice to the University of Leeds. Samples were kept at -80°C prior to analysis.

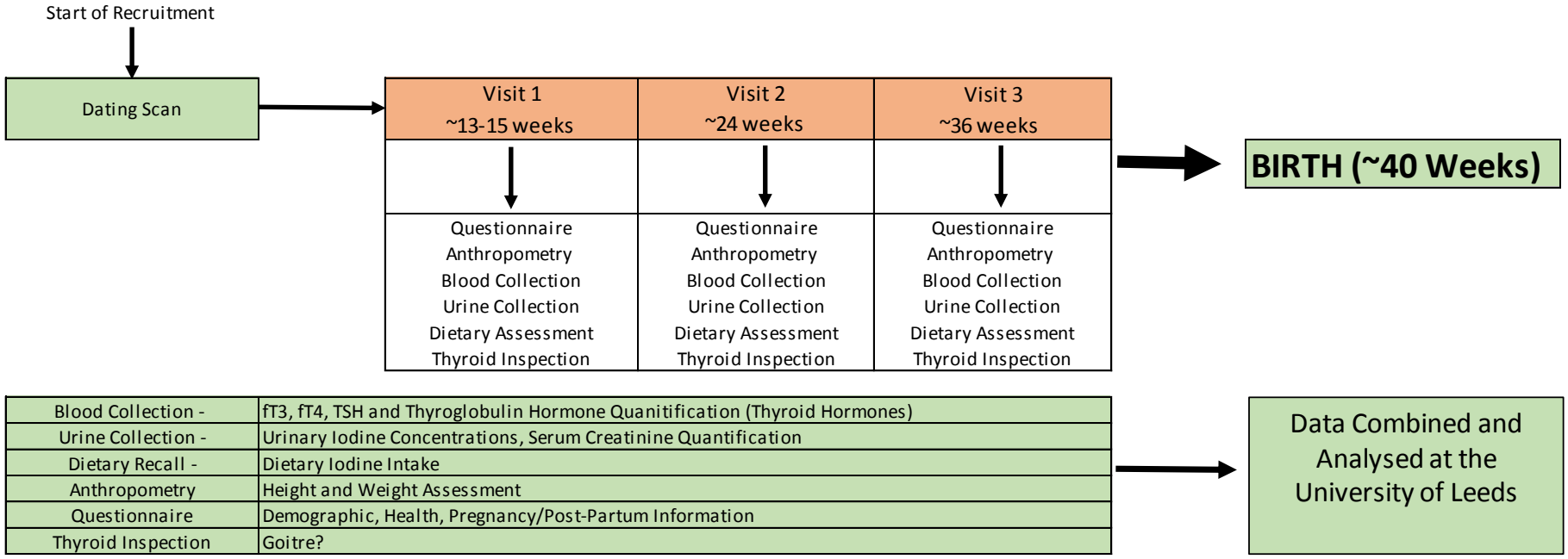


Figure 12: HIBA recruitment timeline. Women were approached at the routine dating scan as per the set criteria (Table 7) and invited to participate in the study. Study visits were undertaken at ~13-15 weeks, ~24 weeks and ~36 weeks gestation. Questionnaires, anthropometric assessments, dietary evaluation and blood and urine samples were administered/collected at each visit.

2.2 Laboratory Procedures

2.2.1 Inductively Coupled Plasma Mass Spectrometry

ICP-MS is a robust technique which works using the high temperatures of a plasma torch with a mass spectrometer to determine elemental concentrations in chemical, environmental and biological samples. First used in the 1980's, it has gained acceptance in the laboratory environment due to its low detection limits (parts per trillion) and high sample throughput (Wolf, 2005). Both UIC and serum selenium concentrations were determined using this method at the School of Earth and Environment, University of Leeds. (Thermo iCAP Q, Liverpool, UK).

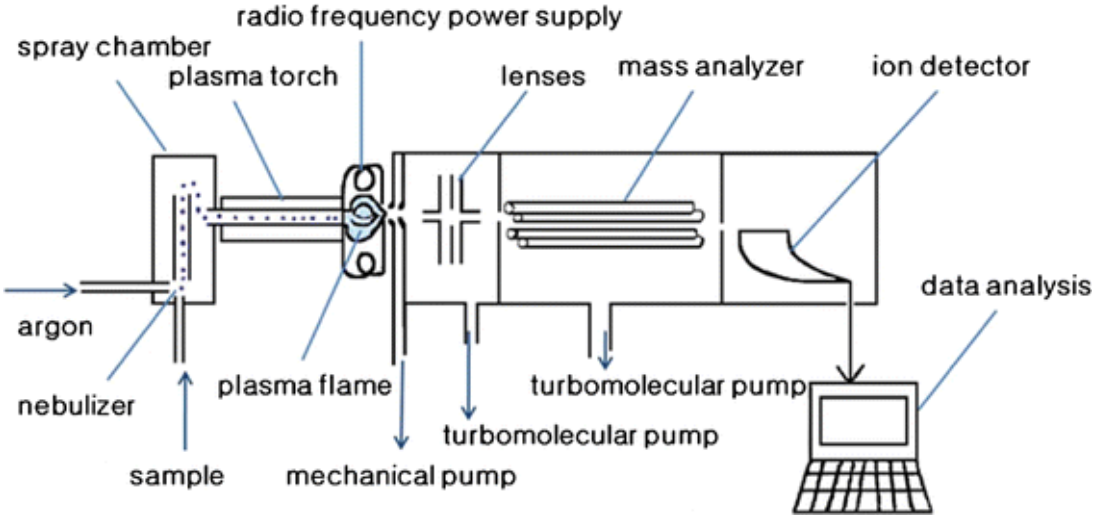


Figure 13: Schematic diagram of the ICP-MS.. Samples are introduced into the ICP-MS system via the autosampler. They are carried by the argon stream via a peristaltic pump to the nebulizer where it is converted from liquid to aerosol in the spray chamber. Aerosol from the spray chamber enters the plasma torch where it is atomised. Further travel through the torch causes atoms to absorb energy until they are eventually ionised. Ions are introduced into the mass analyser where they are separated based on their mass to charge ratio (m/z) (Wolf, 2005; Bazilio and Weinrich, 2012). Figure adopted from Ha et al. (2011).

2.2.1.1 Serum Selenium Method Development

Serum selenium method development was based on the protocol from the 'Centers for Disease Control and Prevention (CDC) Environmental Health Method: Laboratory Procedure Manual for Copper, Selenium and Zinc (DLS 3006.8-02)'. ICP-MS (Thermo iCAP Q, Liverpool, UK) was used for all analyses. Selenium was determined at mass-to-charge ratio (m/z) = 77 (^{77}Se) and m/z = 78 (^{78}Se). ^{80}Se was not used due to significant interference from dimerization of argon from the plasma torch (^{40}Ar^{40}Ar). The instrument was run in kinetic energy discrimination mode using helium as a collision gas. Instrument performance was established before each use by tuning the instrument and running a quality control solution. Two external serum certified reference materials (CRM) with known concentrations of selenium were purchased from Seronorm™ (L1 Lot#: 1309438; L2 Lot#: 1309416) and used during assay development. Quantification limits were determined by running a blank sample for a total of six times to obtain an experimental quantification limit of 17.3 parts per billion (ppb). Repeatability was determined by running the external CRM for a total of six times to calculate the 95% CI.. Results showed the uncertainty at the 95% CI was 3.63%. Reproducibility was determined by repeating the previous experiment 6 times over 4 days. Spike recoveries obtained a 98.9% recovery with a spike of +5 ppb and 108% recovery with a spike of 50 ppb; within the 90-110% acceptable range. CRM results demonstrated that the method was acceptable to be taken forward for sample analysis.

2.2.1.2 Serum Selenium Protocol

Patient samples, calibration standards and quality controls were diluted 1:30 prior to analysis with diluent (10 µg/L gallium internal standard, 0.2% volume/volume double distilled nitric acid, 1% ethyl alcohol and 0.01% Triton X-100; all Sigma Aldrich, UK), deionised H_2O and base serum (Lot# BRH1374677, Seralabs, West Sussex, UK) as per Table 8. Calibration standards were spiked with selenium at the following levels; 30, 120, 210, 300, 1200, 2100 and 3000 ppb with a selenium standard (Lot# RT564, Sigma Aldrich, UK). Quality Controls (QC) were created in house by spiking selenium standard into base serum at 1500 ppb and 150 ppb (calculations not included). External QC material was provided from Seronorm™, UK (L1-2; Lot# 1309438 and 1309416, respectively). For extra validation, study samples were run alongside samples with known selenium concentrations provided by the United Kingdom National External Quality Assessment Service (UK NEQAS).

Sample	Diluent (µL)	H_2O (µL)	Working Standard (µL)	Base Serum (µL)	QC Serum (µL)	Seronorm™ (µL)	Participant Sample (µL)	UK NEQAS Sample (µL)
Participant Samples	4200	150	-	-	-	-	150	-
In House QC	4200	150	-	-	150	-	-	-
Calibration Standards	4200	-	150	150	-	-	-	-
Serum Blank	4200	150	-	150	-	-	-	-
External QC	4200	150	-	-	-	150	-	-
Aqueous Blank	4200	300	-	-	-	-	-	-
UK NEQAS Samples	4200	150	-	-	-	-	-	150

Table 8: Serum selenium sample preparation. Selenium samples were prepared as per the following table fresh on the day of analysis. Participant samples, QC samples and calibration standards and UK NEQAS were allowed to thaw to an ambient temperature prior to use. Samples were vortexed before ICP-MS sample loading.

2.2.1.3 Urinary Iodine Development and Analysis

Details of the UIC development process using ICP-MS is detailed in Snart et al. (2019). The method to determine urinary iodine concentration was based on the accredited 'CDC Ensuring the Quality of Urinary Iodine Procedures (EQUIP)' protocol. Samples, calibration standards, and QC samples were diluted 1:10 prior to sample analysis with diluent (1% tetramethylammonium hydroxide, 0.01% Triton X-100 and 10 µg/L tellurium (all Sigma Aldrich, UK)), deionised H_2O and base urine as seen in Table 9. Sample concentrations were calculated against a urine matched standard calibration curve spiked with iodide at the following levels: 0, 5, 10, 40, 70, 100, 400, and 800 µg/L (Sigma Aldrich, UK). Low, medium and high internal QC samples were set at iodine concentrations between 40-75 µg/L, 90-110 µg/L and >100 µg/L, respectively. External CRM Seronorm™ Trace Metal Urine L1 and CDC EQUIP participation were used as external validation measures.

Sample	Diluent (µL)	H_2O (µL)	Working Standard (µL)	Base Urine (µL)	QC Urine (µL)	Seronorm™ (µL)	Participant Sample (µL)
Participant Samples	4000	500	-	-	-	-	500
In house QC	4000	500	-	-	500	-	-
Calibration Standards	4000	-	500	500	-	-	-
Urine Blanks	4000	500	-	500	-	-	-
External QC	4000	500	-	-	-	500	-

Table 9: Urinary iodine sample preparation. Urinary iodine samples were prepared as per the following table fresh on the day of analysis. Participant samples, QC samples and calibration standards were allowed to thaw to an ambient temperature prior to use. Samples were vortexed before ICP-MS sample loading.

2.2.2 Enzyme Linked Immunosorbent Assay

Enzyme Linked Immunosorbent Assays (ELISA) are a specific analytical tool used to detect levels of proteins, hormones and biomarkers. A derivative of radioimmunoassay, ELISA uses the immunological concepts of antigen/antibody complexes with an enzyme-linked signal generator in order to quantify target molecules (Crowther, 2009; Gan and Patel, 2013). Thyroid hormones and selenium biomarkers were quantified using pre-validated commercial ELISA kits

2.2.2.1 Serum Glutathione Peroxidase 3

Human serum GPx3 was quantified using a pre-validated 'sandwich' ELISA kit (Lot number: K4001805, Catalog #: AG-45A-0020YEK-KI01, Adipogen, Switzerland). 5 kit constituents were prepared fresh on the day prior to analysis (human GPx3 standard [64 ng/mL] for calibration standard preparation, wash buffer 1x, ELISA buffer 1x, detection antibody and horse radish peroxidase (HRP)-labelled streptavidin). Human GPx3 standard was reconstituted with 1 mL deionised water and thoroughly mixed prior to standard preparation. Standards were produced according to Table 10. Wash buffer 1x was produced by diluting wash buffer 10x, 10-fold with deionised water. ELISA buffer 1x was produced by diluting ELISA buffer 10x, 10-fold with deionised water. The detection antibody was diluted 1000-fold prior to analysis with 1x ELISA buffer and was not stored for future use. HRP-labelled streptavidin was reconstituted with 100 µL of ELISA Buffer 1x and thoroughly mixed. The reconstituted HRP-labelled streptavidin was further diluted 200-fold and used within the assay. Participant serum samples were centrifuged for 6 minutes at 4600 rpm and diluted 1:1000 prior to loading with ELISA buffer 1x. 100 µL of standards and samples were loaded onto the plate as per the plate layout in duplicate and incubated with gentle shaking for 1 hour at 37°C. Following incubation, wells were aspirated and washed three times with wash buffer 1x using an automated plate washer (Aspect Scientific, Cheshire, UK). 100 µL of detection antibody was added to the empty plate and incubated with gentle shaking for 1 hour at 37°C. The wash step was repeated and 100 µL of diluted HRP-labelled streptavidin was added to the wells and left to incubate with gentle shaking for 1 hour at 37°C. The wash step was repeated and 100 µL of tetramethylbenzidine (TMB) was added to the empty wells and shielded from light for 20 minutes at room temperature. 50µL of stop solution was added to all wells and the plate was read using a spectrophotometer at 450nm (Thermo Fisher MultiSkan Go!, UK). Optical densities were proportionate to the amount of sample in each well. Mean values were calculated and the blank optical density was subtracted prior to the production of a 4-parameter logistical curve.

Standard	Spike Solution	Volume of Spike Solution (µL)	Diluent (µL)	Concentration (ng/mL)
A	64ng/mL Stock Standard	400	400	32
B	Standard A	400	400	16
C	Standard B	400	400	8
D	Standard C	400	400	4
E	Standard D	400	400	2
F	Standard E	400	400	1
G	Standard F	400	400	0.5
H	-	-	400	0

Table 10: Standard concentrations for GPx3. Standards for the GPx3 calibration curve were produced between the concentration ranges of 0-32 ng/mL using ELISA buffer 1x as diluent. Samples were prepared fresh on the day and were not stored for future use.

Standard	Spike Solution	Volume of Spike Solution (µL)	Diluent (µL)	Concentration (ng/mL)
A	50ng/mL Stock Standard	400		50
B	Standard A	400	400	25
C	Standard B	400	400	12.5
D	Standard C	400	400	6.25
E	Standard D	400	400	3.125
F	Standard E	400	400	1.562
G	Standard F	400	400	0.781
H	-	-	400	0

Table 11: Standard concentrations for SEPP1. Standards for the SEPP1 calibration curve were produced between the concentration ranges of 0-50 ng/mL using the sample dilution buffer as diluent. Samples were prepared fresh on the day and were not stored for future use.

2.2.2.2 Serum Selenoprotein P

Human serum SEPP1 was quantified using a pre-validated 'sandwich' ELISA kit (Cat#: EH1943, Lot: H1943C122, Wuhan Fine Biotech Co Ltd). Four constituents were prepared fresh on the day of analysis (calibration standards from lyophilised standard [50 ng/mL], 1x wash buffer, biotin-labelled antibody working solution and HRP-streptavidin conjugate). The 50 ng/mL lyophilised standard was reconstituted with 1mL sample dilution buffer and left for 10 minutes prior to thorough mixing. Standards were prepared as per Table 11. Wash buffer concentrate was diluted 1:25 with deionised water to create 1x wash buffer. Both the biotin labelled antibody working solution and HRP-streptavidin conjugate were diluted 1:100 prior to use in the assay (120µL of solution into 11,880µL of antibody/ HRP-streptavidin conjugate dilution buffers, respectively). Prior to sample loading, the pre-coated plate was washed a total of 2 times with 350 µL of 1x wash buffer using an automated plate washer (Aspect Scientific, Cheshire, UK). Serum samples were centrifuged for 6 minutes at 4600 rpm and diluted 1:1000 prior to loading. 100 µL of calibration standards and samples were loaded onto the plate in duplicate and incubated with gentle shaking for 90 minutes at 37°C. After incubation, the plate was washed 2 times with 350 µL of 1x wash buffer and tapped on absorbent paper to remove any excess buffer. 100 µL of 1:100 biotin-labelled antibody working solution was subsequently added to the plate and left to incubate with gentle shaking for 60 minutes at 37°C. After incubation, the plate was washed a further 3 times with buffer being left to soak in the wells for 1 minute between cycles. 100 µL of 1:100 HRP-streptavidin conjugate working solution was added to the plate and incubated with gentle shaking for 30 minutes at 37°C. After incubation, the cover film was removed and washed 5 times with a 90 second soak in each well after each wash cycle. After the final aspiration, 90 µL of TMB was added to each well and left to incubate for approximately 10 mins in darkness at 37°C. 50 µL of stop solution was added to stop the reaction and the plate was read immediately at 450nm by a spectrophotometer (Thermo Fisher MultiSkan Go!, UK). Optical densities were proportionate to the amount of sample in each well. Mean values were calculated and the blank optical density was subtracted prior to the production of a 4-parameter logistical curve.

2.2.2.3 Serum Free Thyroxine

Human serum fT4 was quantified using a competitive ELISA kit (Lot number: X17-302, Biovendor, Czech Republic) as per the product data sheet (Cat No: RCD015R). The majority of constituents within the kit were ready to use - two constituents required preparation (fT4 HRP conjugate and wash buffer). The HRP conjugate was diluted 1:50 before use in a glass vial and vortexed thoroughly before use. Wash buffer concentrate was diluted 1:10 prior to use with deionised water and inverted numerous times to make sure the concentrate was thoroughly mixed. Seronorm™ Immunoassay Lyo L1-3 (Lot's# 1510466, 1510478, 1510479 respectively; Seronorm™, UK) was used as the external QC. Standard curve concentrations are listed in Table 12. Serum samples were centrifuged for 6 minutes at 4600 rpm prior to loading. 25 µL of standards, QC and participant samples were added into each well as per the plate layout in duplicate. 100 µL of HRP conjugate was added to each well and incubated at 37°C for 1 hour. After incubation, wells were washed 3 times with 300 µL of wash buffer using an automated plate washer (Aspect Scientific, Cheshire) and tapped to remove excess solution. 150 µL of TMB was added to the empty wells and left to incubate at 37°C. 50 µL of stop solution was added after 15 minutes and the plate was read by the spectrophotometer at 450nm (Thermo Fisher MultiSkan Go!, UK). A 4-parameter logistical curve was used to interpret calibration and sample concentrations.

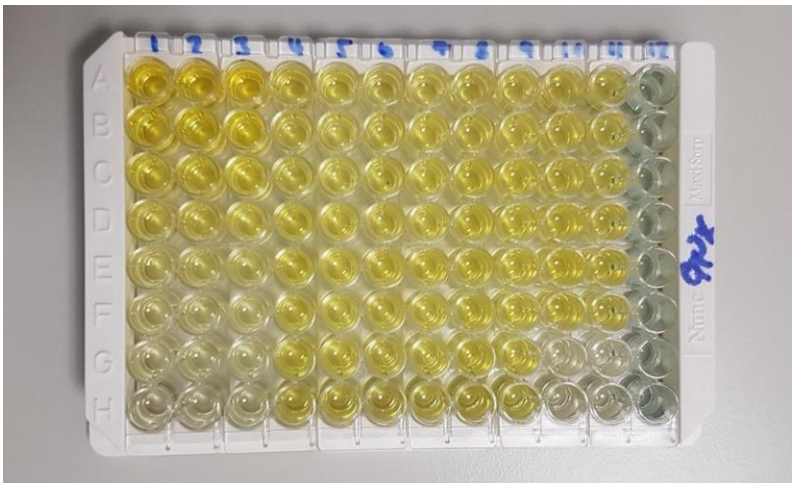


Figure 14: ELISA assay completion. The final step of the ELISA protocol ends the assay via the addition of sulphuric acid. The plate is then read at 450nm by the spectrophotometer where the optical densities are calculated for standards, QC samples and patient samples. This step is applicable to all assays.

2.2.2.4 Serum Free Triiodothyronine

Human serum fT3 was quantified using a competitive ELISA kit (Lot number: X17-303, Biovendor, Czech Republic) as per the product data sheet (Cat No: RCD014R). The majority of constituents within the kit were ready to use - two constituents required preparation prior to analysis (fT3 HRP conjugate and wash buffer). The HRP conjugate was diluted 1:50 before use in a glass vial and vortexed thoroughly before use. Wash buffer concentrate was diluted 1:10 prior to use with deionised water. Seronorm™ Immunoassay Lyo L1-3 (Lot's# 1510466, 1510478, 1510479 respectively, Seronorm™ UK) were used as an external QC. Standard curve concentrations are listed in Table 13. Serum samples were centrifuged for 6 minutes at 4600 rpm prior to loading. 25 µL of standards, QC samples and participant samples were added into each well as per the plate layout in duplicate. 100 µL of HRP conjugate was added to each well and incubated at 37°C for 1 hour. After incubation, wells were washed 3 times with 300 µL of wash buffer using an automated plate washer (Aspect Scientific, Cheshire) and tapped to remove excess solution. 150 µL of TMB was added to the empty wells and left to incubate at 37°C. 50 µL of stop solution was added after the15 minute incubation and read by the spectrophotometer at 450nm (Thermo Fisher MultiSkan Go!, UK). A 4-parameter logistical curve was used to interpret calibration and sample concentrations.

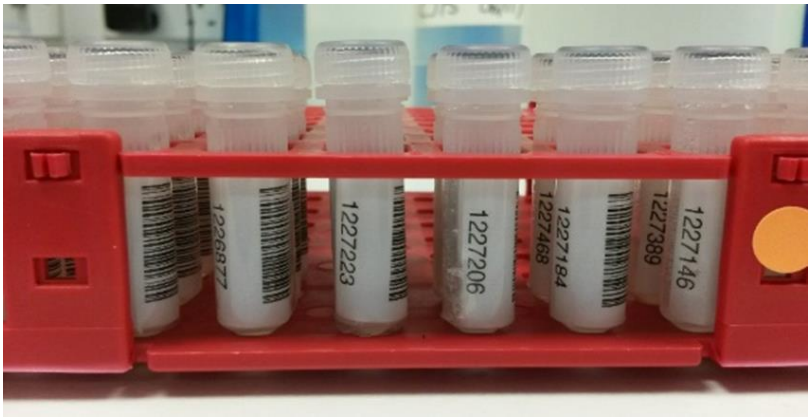


Figure 15: HIBA cohort samples. Samples were thawed and centrifuged prior to analysis. Each sample was identified by a unique, seven digit number as seen in Figure 15. Samples were returned to the -80ºC freezer within a 2 hour window to maintain sample integrity.

2.2.2.5 Serum Thyroid Stimulating Hormone

Human serum TSH was quantified using a competitive ELISA kit (Lot number: X17-301, Biovendor, Czech Republic) as per the product data sheet (Cat No: RCD028R). The majority of constituents within the kit were ready to use - two constituents required preparation prior to use (TSH HRP conjugate and the wash buffer). The HRP conjugate was diluted 1:50 prior to use in a glass vial. Wash buffer concentrate was diluted 1:10 prior to use with deionised water. Seronorm™ Immunoassay Lyo L1-3 (Lot's# 1510466, 1510478, and 1510479 respectively, Seronorm™ UK) was used as an external QC. Standard curve concentrations are listed in Table 14. Serum samples of the participants were centrifuged for 6 minutes at 4600 rpm prior to loading. 50 µL of standards, QC samples and participant samples was added into each well as per the plate layout in duplicate. 100 µL of HRP conjugate was added to each well and incubated at room temperature for 1 hour shaking at 400 rpm. After incubation, wells were washed 3 times with 300 µL of wash buffer using an automated plate washer (Aspect Scientific, Cheshire) and tapped to remove excess solution. 150 µL of TMB was added to wells and left to incubate at room temperature for 15 minutes, shaking at 400 rpm. 50 µL of stop solution was added after the 15 minute incubation and read by the spectrophotometer at 450 nm (Thermo Fisher MultiSkan Go!, UK). A 5-parameter logistical curve was used to interpret calibration and sample concentrations.

Figure 16: Thermo Scientific MultiSkan GO! Spectrophotometer. Optical densities for each ELISA assay were quantified using the spectrophotometer at the end of each experiment. Results were exported and used for calculating calibration curves and determining sample concentrations.

Standard	Concentration (pg/mL)
A	0
B	2
C	6
D	20
E	80

Table 12: Standard concentrations for fT4. Standards for the fT4 calibration curve were quantified between the concentration ranges of 0-80 pg/mL. Standards were pre-prepared and not stored after use.

Standard	Concentration (pg/mL)
A	0
B	2
C	4
D	8
E	16
F	40

Table 13: Standard concentrations for fT3. Standards for the fT3 calibration curve were quantified between the concentration ranges of 0-40 pg/mL. Standards were pre-prepared and not stored after use.

Standard	Concentration (µIU/mL)
A	0
B	0.2
C	1
D	5
E	15
F	30

Table 14: Standard concentrations for TSH. Standards for the TSH calibration curve were quantified between the concentration ranges of 0-30 µIU/mL. Standards were pre-prepared and not stored after use.

2.2.2.6 Serum Thyroglobulin

Human serum thyroglobulin was quantified using a pre-validated commercial 'sandwich' ELISA kit (Lot number: GR3221643, Abcam, UK). 5 kit constituents were prepared fresh on the day (calibration standards, 1x assay diluent B, 1x wash solution, 1x biotinylated thyroglobulin detection antibody and HRP streptavidin solution). Thyroglobulin lyophilised standard was reconstituted using 400 µL of assay diluent C before centrifugation to ensure thorough mixing. Standards were serially diluted as per Table 15. 1x assay diluent B was prepared by diluting 10 mL 5x assay buffer B into 40 mL deionised water. 25 mL of 20x wash buffer was diluted in 475 mL of deionised water to produce 1x wash solution. Prior to reconstituting the biotinylated thyroglobulin detection antibody, the vial was centrifuged to ensure all material was in the bottom of the tube. 100 µL of 1x assay diluent B solution was added and mixed gently using a pipette. This was then diluted 80-fold (150 µL into 11,850 µL using1x assay diluent B) and used within the assay. The HRP streptavidin concentrate was centrifuged and pipette mixed prior to use. For use in the assay, it was diluted 600-fold (20 µL into 11,980 µL of 1x assay diluent B). Serum samples were centrifuged for 6 minutes at 4600 rpm prior to loading onto the plate. 100 µL of standards, samples and QCs were added onto the plate as per the plate layout and incubated with gentle shaking for 2.5 hours at room temperature. Following incubation, wells were washed using an automated plate washer (Aspect Scientific, Cheshire) 4 times with 300 µL of 1x wash solution. The plate was tapped on absorbent paper to remove any excess solution. 100 µL of 1x biotinylated thyroglobulin detection antibody was added into each well and incubated with gentle shaking for 1 hour at room temperature. Following further washing (as described above), 100 µL of HRP-streptavidin solution was added to each well and incubated with gentle shaking for 45 minutes at room temperature. Following the final wash step, 100 µL of TMB solution was added to all wells and incubated with gentle shaking for 30 minutes at room temperature shielded from light. 50 µL of stop solution was added in the final step to stop the reaction. The plate was read using a Spectrophotometer (Thermo Fisher MultiSkan Go!, UK). Mean values were calculated and the blank optical density was subtracted prior to the production of a 4-parameter logistical curve.

Standard	Spike Solution	Volume of Spike Solution (µL)	Diluent (µL)	Concentration (ng/mL)
A	Lyophilised thyroglobulin standard (50 ng/mL)	-	400	50
B	Standard A	200	300	20
C	Standard B	200	300	8
D	Standard C	200	300	3.2
E	Standard D	200	300	1.28
F	Standard E	200	300	0.512
G	Standard F	200	300	0.205
H	-	-	300	0

Table 15: Standard concentrations for thyroglobulin. Standards for the thyroglobulin calibration curve were quantified between the concentrations ranges of 0-50 ng/mL and prepared fresh on the day. Standards were not stored for future use.

2.3 Statistical Analysis

Statistical analysis was performed using STATA Standard Edition v13.1. Questionnaire data, dietary data and biomarker concentration databases from the first three time points (~13-15 weeks, ~24 weeks and ~36 weeks) were imported and merged from Microsoft® Excel. Prior to correlation and regression analysis, all biomarkers were assessed for normality using histogram plots and Schapiro-Wilks analysis (results and plots not displayed). Biomarkers that did not have a normal distribution had corresponding concentrations log transformed before further analysis. Differences of the mean were assessed using either an independent t-test or Mann Whitney-U test (depending on distribution). Pearson's correlation was used to assess linear relationships between the following variables:

1. Serum selenium and GPx3/SEPP1/iodine/TSH/fT3/fT4/thyroglobulin/dietary selenium and birthweight
2. Iodine and fT4/fT3/TSH and thyroglobulin.

Moving average plots were created in GraphPad Prism v7.0 to track mean concentrations over time. One-way repeated measure ANOVA with Greenhouse-Geisser correction was performed in STATA to test for significant changes over time. This method was used to take into consideration that several concentration measurements were taken within-individual at differing time points. Significance is indicated if there are differences in concentration between at least two of the time points. Sphericity assumes variance of the differences between-visits are constant, however, in this instance, the requirement was not met. To take into account this violation, Greenhouse-Gessier correction was used for all results generated from the ANOVA. fT3/fT4 ratios were determined by calculating mean values of fT3, dividing them by the mean values of fT4. This ratio will determine the normal functioning of the DIO. Repeated measures linear regression with and without adjustment for confounders (age, smoking, body mass index (BMI), alcohol consumption, all supplementation usage, ethnicity and where applicable, UIC and selenium) was performed in STATA to model associations between variables. We were able to take into account the repeated measures using a sandwich estimation allowing for the similarity of results 'within-person' and any possible heteroscedasticity. All results are subject to a 5% significance level and 95% CI.

Results

3.1 Maternal Characteristics

Participant demographics were collated using the questionnaire data (see section 2.1.2) and are displayed in Table 16. The mean (standard deviation (SD)) age of the women in the study was 30 (5.8) years. Average weight and BMI increased consistently throughout trimesters with a 9.8 kg increase and a 4 kg/m^2 increase from visit one to visit three, respectively. For 37% of the mothers, this was their first pregnancy. Just over half of the women were Pakistani and 35.7% were White-British. Either one or two women reported smoking and alcohol consumption at each visit (1.4-2.9%). Vitamin supplementation was extremely high, peaking at the first visit with 97.1%. 79.4% of these supplements taken at this time point were multivitamins for pregnancy, with just over half of these confirmed to contain selenium and iodine.

	n	Mean (SD)
Maternal Age (years)	69	30.4 (5.8)
Maternal Weight (kg)		
1st Visit	69	70 (12.6)
2nd Visit	59	75.2 (13.7)
3rd Visit	63	79.8 (13.5)
Maternal Height (cm)	65	160.4 (7.0)
BMI (kg/m^2)		
1st Visit	65	27.4 (5.3)
2nd Visit	55	29.6 (5.4)
3rd Visit	59	31.4 (5.3)
	n	n (%)
Parity (%)	70	
0	26	37.1
1	23	32.9
2	14	20.0
3+	7	10.0
Previous Miscarriage (%)	70	
Yes	26	37.1
No	44	62.9
Smoking (%)	70	
1st Visit	1	1.4
2nd Visit	1	1.4
3rd Visit	2	2.9
Ethnicity (%)	70	
White British	25	35.7
Pakistani	37	52.9
Other[1]	8	11.4
Alcohol Consumption (%)	70	
1st Visit	1	1.4
2nd Visit	2	2.9
3rd Visit	1	1.4
Vitamin Supplementation (%)	70	
1st Visit	68	97.1
2nd Visit	60	85.7
3rd Visit	59	84.3

Table 16: Maternal characteristics. Descriptive statistics of the women used within the analysis. Continuous variables are reported as Mean (SD) and categorical variable are reported as n (%) [1]Other Ethnicities include Bangladeshi, Indian, Polish and Romanian. Total sample size = 70. Small amounts of missing data accounts for $n \neq 70$ for some variables

3.2 Ethnicity Analysis

The bi-ethnic cohort allowed for a small analysis of the two main ethnic groups (White-British and Pakistani) to determine differences in selenium, iodine and their corresponding biomarker concentrations. The median concentration (interquartile range (IQR)) of each biomarker across all three time points are displayed in Table 17. Only iodine status was found to be significantly different between the two groups.

Biomarker	White-British	Pakistani
Selenium (µg/L)	64.9 (15.3)	63.6 (14.1)
Iodine (µg/L)***^**	88.9 (120)***^**	132.2 (144)***^**
SEPP1 (ng/mL)	3784 (1144)	4017 (1018)
GPx3 (ng/mL)	6972 (2382)	6776 (1712)
fT3 (pg/mL)	2.3 (0.66)	2.1 (1.26)
fT4 (pg/mL)	8.7 (3.07)	8.7 (2.70)
TSH (µIU/mL)	1.4 (0.85)	1.2 (1.12)
Thyroglobulin (ng/mL)	5.1 (6.80)	3.7 (11.4)

Table 17: Biomarker concentrations by ethnicity. Median (IQR) values for each biomarker is shown in the table above. Independent t-tests were performed on normally distributed data (iodine and thyroglobulin not normally distributed - Mann Whitney U test performed). ***** signifies $p<0.05$, ^outliers excluded >500µg/L (n=3).

3.3 Selenium, Glutathione Peroxidase 3 and Selenoprotein P

Table 18 shows the median (IQR) values at each visit for the three listed biomarkers. Whilst median serum selenium and GPx3 concentrations were seen to fall throughout pregnancy (from 71.4 to 57 µg/L selenium; 7076 to 6443 ng/mL GPx3), SEPP1 concentrations increased steadily from 3750 µg/L in visit one to 4300 ng/mL in visit three. 94.2% of individuals were below the 90 µg/L selenium threshold for optimal GPx3 expression at the first visit - this value increased to 100% of participants at the third visit (Thomson et al., 1977). Figure 17 and Figure 18 shows the relationship between selenium and the selenium containing enzymes, GPx3 and SEPP1. In Figure 17, GPx3 was found to be positively correlated with serum selenium concentration across all three visits. Pearson's correlation was performed at each individual time point (Fig 17, A-C) and for all visits (Fig 17, D) with results confirming a moderate relationship between the two biomarkers (Pearson's correlation coefficient Graph D:, r_p=0.59, 95% CI 0.49 to 0.67, p<0.001). Plots showing the mean values (with 95% CI) for GPx3 and selenium at each visit are displayed in Graphs E and F. A decreasing trend in concentrations of GPx3 and serum selenium is apparent as gestation progresses. For both biomarkers, a one-way repeated measure ANOVA with Greenhouse-Geisser correction was performed. It was found that throughout pregnancy, decreases in selenium (F [2,137] = 92.02, p<0.001) and GPx3 (F [2, 138] = 23.35, p<0.001) were statistically significant. Using adjusted and unadjusted repeat measure linear regression models (Table 23), GPx3 was found to be significantly associated with serum selenium status during pregnancy (p<0.001 both unadjusted and adjusted for age, smoking, BMI, alcohol consumption, supplementation usage and ethnicity). For every 1 µg/L increase in serum selenium, there was a 68 ng/mL increase in GPx3 concentration (β = 68, 95% CI 50.1 to 88.0). SEPP1 was negatively correlated with serum selenium (Figure 18) across the duration of pregnancy (Fig 18, A-D). Pearson's correlation was performed for all visits (Fig 18, D) with results confirming a weak to moderate negative relationship (Pearson's coefficient r_p= -0.32, 95% CI -0.44 to -0.20, p<0.001). The moving average plot as seen in Graph E clearly shows a mean rise in SEPP1 concentration from visit 1 to visit 3 and this was statistically significant as per one-way repeated measure ANOVA with Greenhouse-Geisser correction (F [2,132] = 30.57, p<0.001). Using unadjusted and adjusted repeat measure linear regression models, serum selenium was found to be a negative predictor of SEPP1 (p<0.001). For every 1 µg/L increase in serum selenium, SEPP1 decreased by 25 ng/mL (β =-25, 95%CI -36.7 to -13.3).

Biomarker	Visit 1 (n=70)	Visit 2 (n=70)	Visit 3 (n=70)
Serum selenium (µg/L)	71.4 (16.4)	64.2 (12.2)	57.7 (15.3)
Urinary iodine (µg/L)*	103.4 (118.4)	120.8 (116.6)]	112.1 (177.5)
Glutathione peroxidase 3 (ng/mL)	7076.0 (1865.0)	6811.6 (2050.1)	6443.3 (2143.5)
Selenoprotein P (ng/mL)	3749.5 (1016.4)	3761.2 (925.2)	4299.3 (940.3)
TSH (µIU/mL)	1.12 (0.9)	1.35 (1.0)	1.53 (0.9)
fT4 (pg/mL)	9.7 (2.8)	8.2 (2.6)	8.2 (3.4)
Thyroglobulin (ng/mL)	5.6 (10.2)	4.3 (9.4)	4.6 (12.7)
fT3 (pg/mL)	2.4 (0.9)	2.0 (0.8)	2.1 (2.4)

Table 18: Median biomarker concentrations. Serum selenium, urinary iodine, GPx3, SEPP1, TSH, fT4, fT3 and thyroglobulin concentrations by visit. Results given as the median (IQR). *Outliers excluded (>500µg/L, n=3)

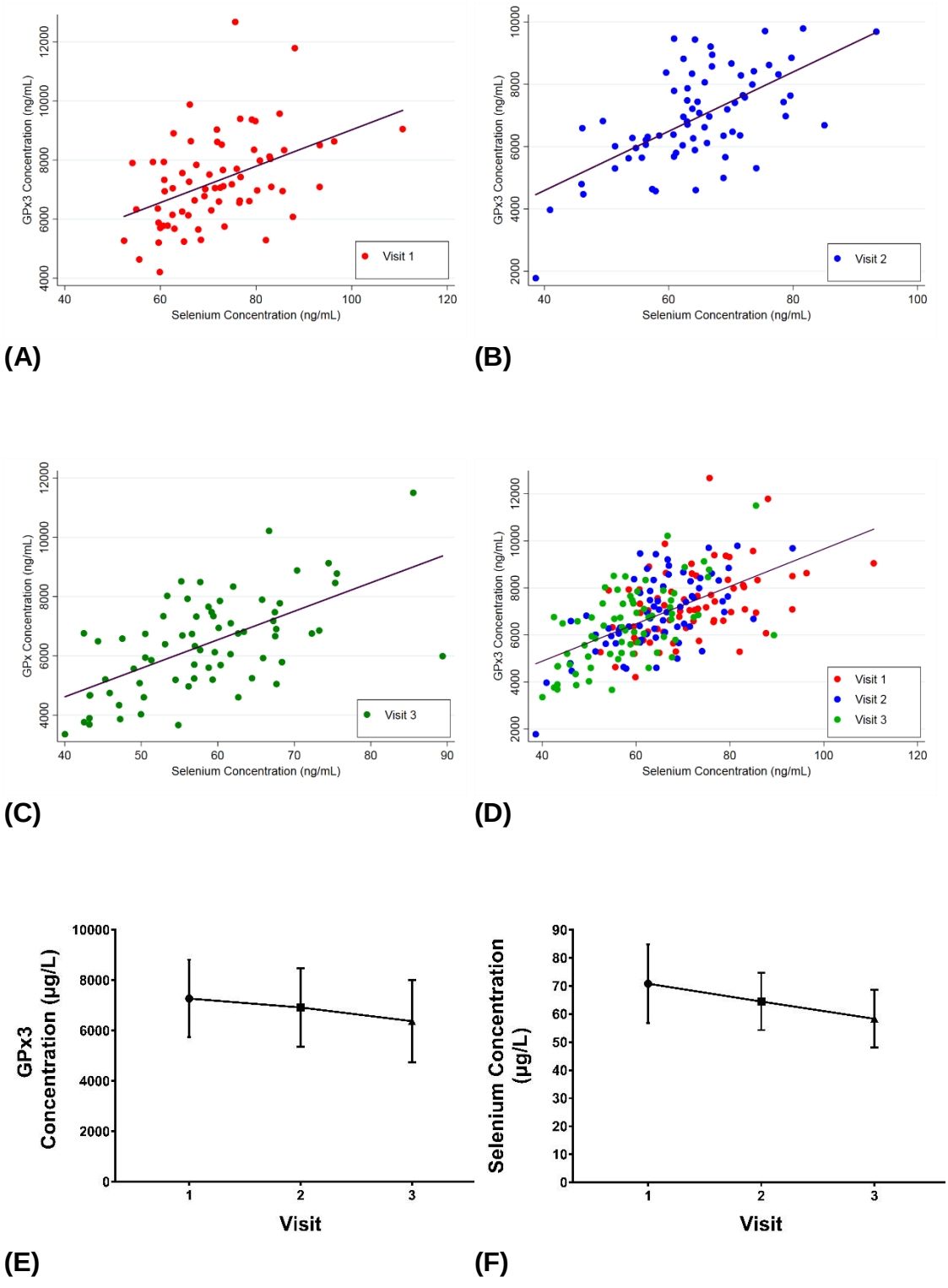


Figure 17: Correlations and moving averages for selenium and GPx3. Graphs A-C show the relationship between serum selenium and GPx3 across each visit (r_p=0.45, 0.63 and 0.61, p<0.001 for all visits, respectively). Graph D shows the combined correlation of all visits throughout pregnancy (p<0.001). Graphs E and F show the mean change over time with 95% CI plotted for each time point.

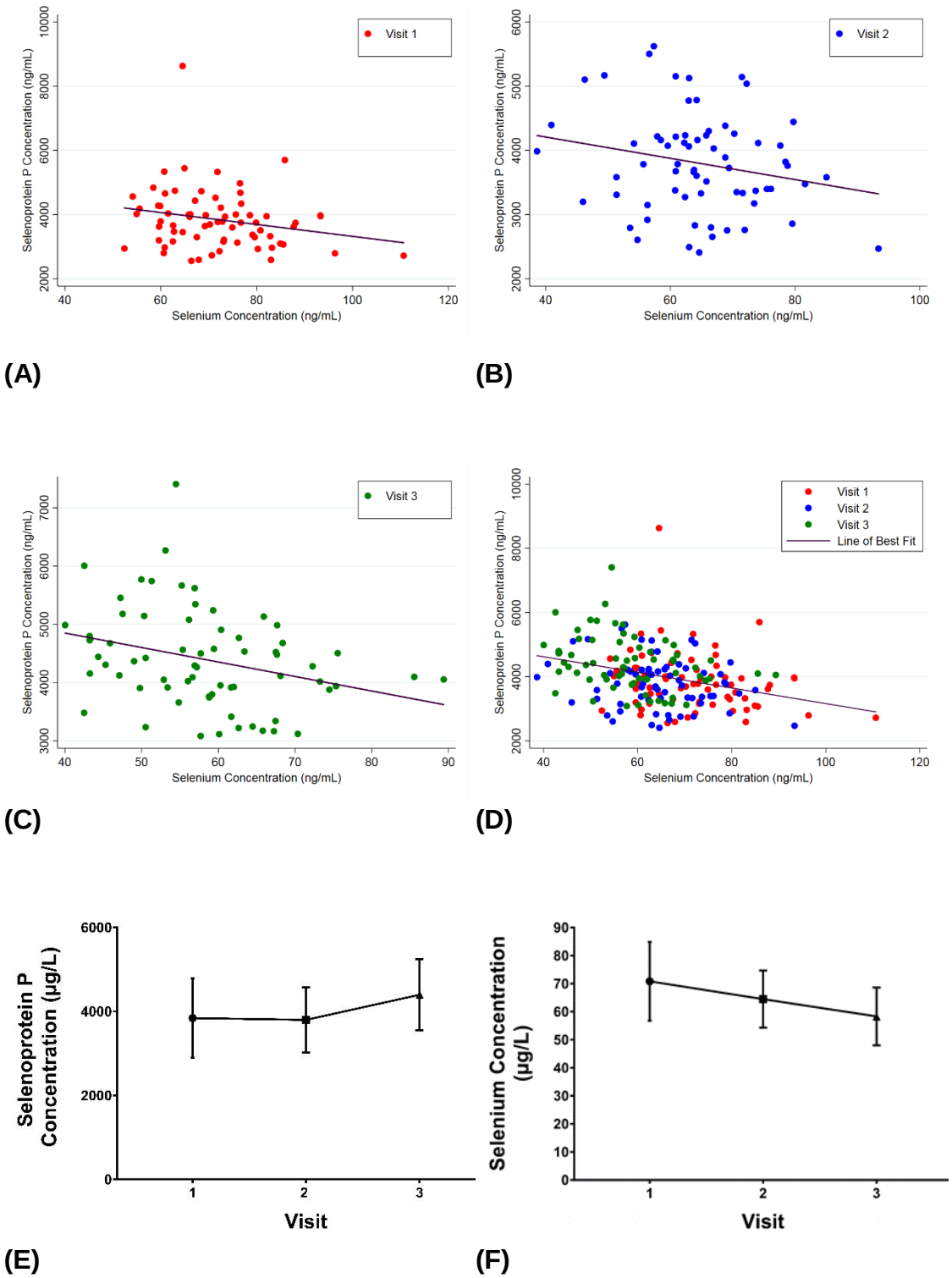


Figure 18: Correlations and moving averages for selenium and SEPP1. Graphs A-C show the relationship between serum selenium and SEPP1 across each visit (r_p= -0.22, -0.22 and -0.31, p<0.001 for visit 3 only). Graph D shows the combined correlation of all visits throughout pregnancy (p<0.001). Graphs E and F show the mean change over time with 95% CI plotted for each time point.

3.4 Urinary Iodine and Thyroid Hormone Associations

Median (IQR) concentrations for iodine, fT4, fT3 TSH and thyroglobulin at each visit is displayed in Table 18. Median iodine concentrations fluctuated throughout pregnancy, remaining consistently below the 150µg/L threshold set by the WHO for UIC during pregnancy. Median TSH levels increased throughout gestation (1.12 µIU/mL to 1.53 µIU/mL), whereas median fT4, thyroglobulin and fT3 decreased from visit one to two. Levels plateaued at the third visit for fT4 and fT3 whilst median thyroglobulin levels increased at the last visit from 4.3(9.4) ng/mL at visit two to 4.6(12.7) ng/mL at visit three. Figure 19 depicts the strength of the relationships between iodine and each corresponding thyroid hormone. Pearson's correlation was performed for fT3 (r_p= -0.07, 95% CI -0.20 to 0.07, p=0.36), fT4 (r_p= 0.08, 95% CI -0.06 to 0.22, p=0.24), TSH (r_p= 0.07, 95% CI -0.07 to 0.21, p= 0.31) and thyroglobulin (r_p=-0.14, 95% CI -0.27 to 0.003, p=0.06). Results indicated that there was no relationship between iodine and fT3, fT4 and TSH; however there was a weak inverse relationship between iodine and thyroglobulin that was very close to statistical significance (p=0.06). Repeat measures linear regression analysis was performed to determine associations between iodine and all four thyroid hormones (Table 19). After adjustment for confounding with age, ethnicity, smoking, BMI, serum selenium concentration, alcohol consumption and vitamin supplementation; no significant associations were found.

	Adjusted Estimates	
Iodine	β coefficient (CI 95%)	*p* value
fT3 (pg/mL)	0.95 (0.82 to 2.33)	0.48
fT4 (pg/mL)	1.40 (0.85 to 2.33)	0.19
TSH (µIU/mL)	1.03 (0.87 to 1.24)	0.67
Thyroglobulin (ng/mL)	0.94 (0.72 to 1.25)	0.70

Table 19: Iodine and thyroid hormone regression analysis. Beta coefficients, 95% CI and *p*-values after repeat measure linear regression between iodine and its corresponding biomarkers. Results for all biomarkers are presented as a ratio due to the use of log transformed data for iodine and thyroglobulin (not normally distributed).

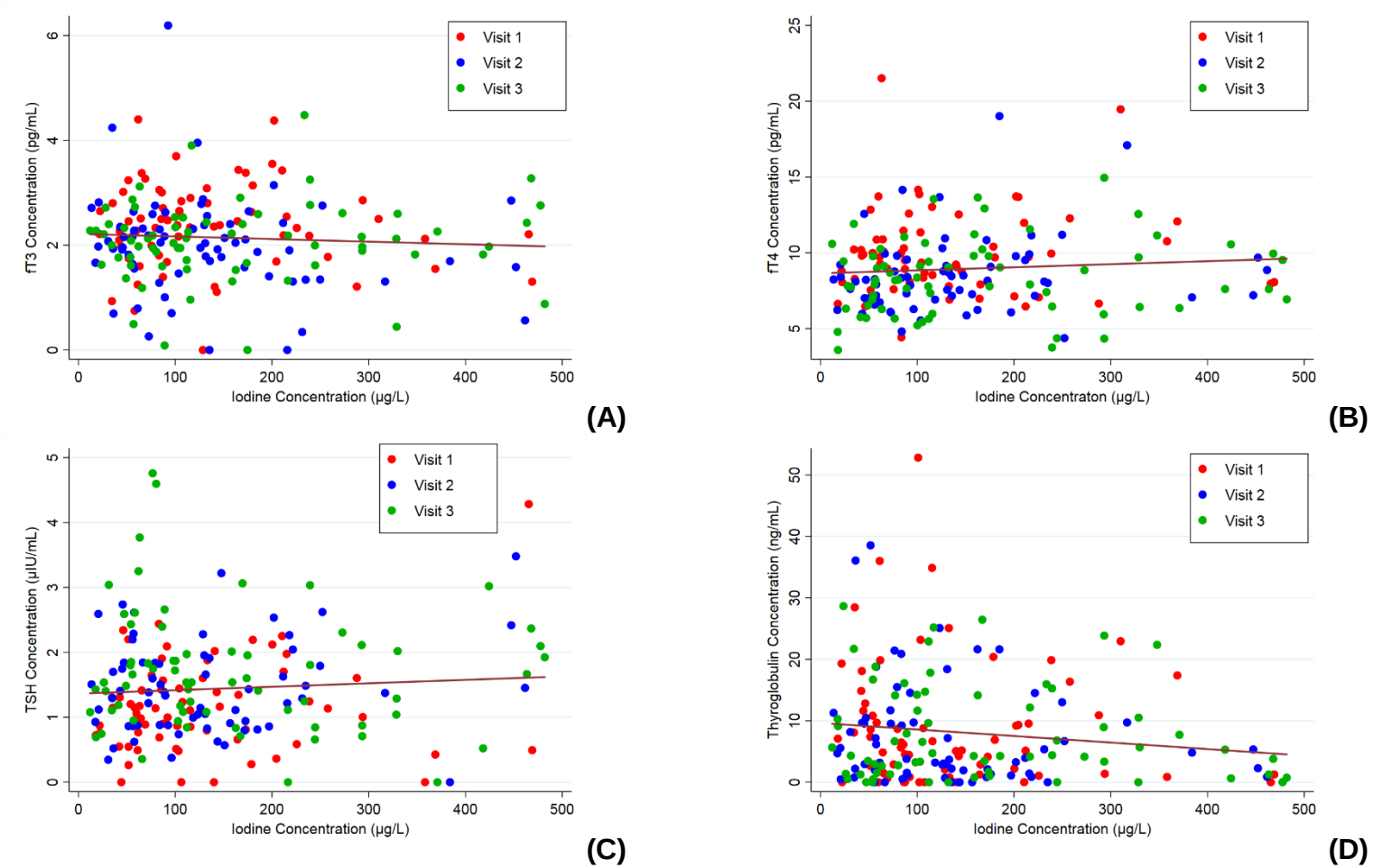


Figure 19: Correlations between iodine and the thyroid hormones. Graphs A-D show the relationship between iodine and fT3 (Graph A), fT4 (Graph B), TSH (Graph C) and thyroglobulin (Graph D) across all time points throughout pregnancy.

3.5 Serum Selenium and Thyroid Hormone Associations

Serum selenium concentration was correlated against each thyroid hormone as seen in Figure 20, Figure 21, Figure 22 and Figure 23. fT3, fT4 and TSH were found to be normally distributed. Thyroglobulin was found not to be normally distributed therefore data for this biomarker was log transformed prior to regression analysis. In each of the figures, Graphs A-C depict selenium and its correlation with each thyroid hormone at visits one to three. Graph D shows selenium concentration with each biomarker for all 70 participants at all visits. Pearson's correlation using all time points was calculated for all hormones (Graph D). fT3 (r_p= 0.06, 95% CI -0.08 to 0.19, *p*=0.41) and TSH (r_p= -0.08, 95% CI -0.21 to 0.06, *p*=0.27) did not show a relationship with selenium. fT4 (r_p= 0.13, 95% CI -0.01 to 0.26, *p*=0.07), and thyroglobulin (r_p= 0.12, 95% CI -0.03 to 0.26, *p*=0.12) displayed a small positive correlation with serum selenium concentration, however no result was statistically significant. Moving average plots with 95% CI (Graphs E) mimic the trends seen in Table 18. Wide confidence intervals as seen for thyroglobulin and TSH are indicative of a small population size and are normal given the study design. One way repeated measure ANOVA with Greenhouse-Geisser correction was performed to test for significant differences between at least two of the mean values for each hormone. A significance difference in mean concentration for all hormones was found, except for thyroglobulin (Table 20). Table 21 shows the ratios between fT3 and fT4 at each visit. Results demonstrated that across pregnancy, the concentrations of the thyroid hormones remained consistent. This indicates that DIO expression was sufficient for the conversion of fT4 to fT3

Repeat measure linear regression was performed for all four thyroid hormones. Beta coefficients, confidence intervals and *p* values are displayed in Table 22. As thyroglobulin was not normally distributed, beta coefficients are displayed as a ratio. Adjusting for age, smoking, BMI, alcohol consumption, UIC, supplementation usage and ethnicity, analysis showed that serum selenium concentration was not a statistically significant predictor of fT3 (β= 0.008, CI 95% -0.01 to 0.02, *p*=0.31), fT4 (β= 0.03, CI 95% -0.02 to 0.08 *p*=0.23), TSH (β= 0.005, CI 95% -0.01 to 0.02, *p*=0.52) and thyroglobulin (β= 1.01, CI 95% 0.99 to 1.03, *p*=0.30). For every 1µg/L increase in serum selenium, there were minimal increases in concentrations of fT3 (0.008 pg/mL), fT4 (0.03 pg/mL), TSH (0.005 µIU/mL) and Thyroglobulin (1%).

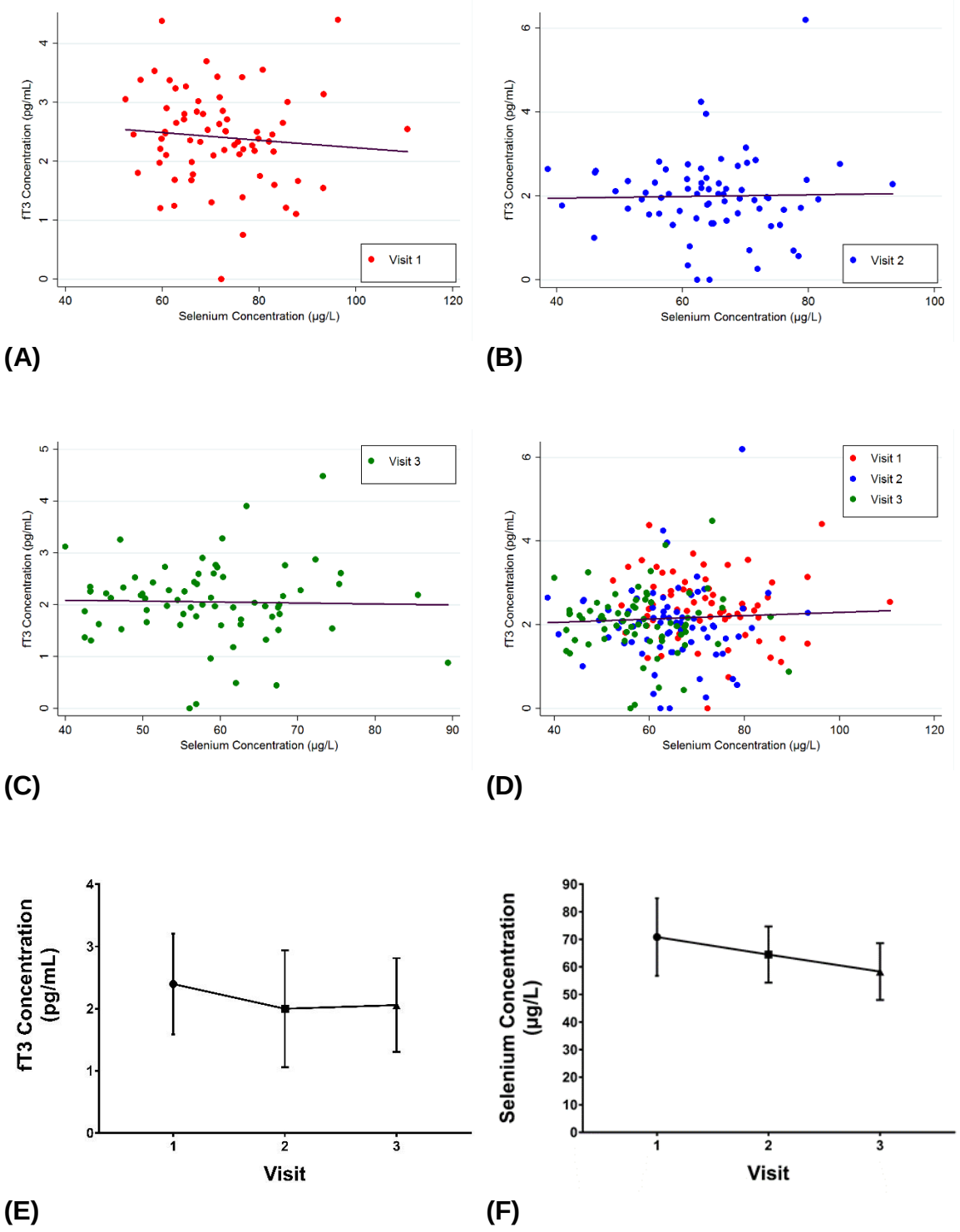


Figure 20: Correlations and moving averages for selenium and fT3. Graphs A-C show the relationship between selenium and fT3 across each visit (r_p= -0.09, 0.02 and -0.02, p>0.05 for all visits, respectively). Graph D shows the combined correlation of all visits throughout pregnancy (r_p=0.05, p=0.41). Graphs E and F display how mean fT3 and selenium concentrations change over time with 95% CI plotted for each time point.

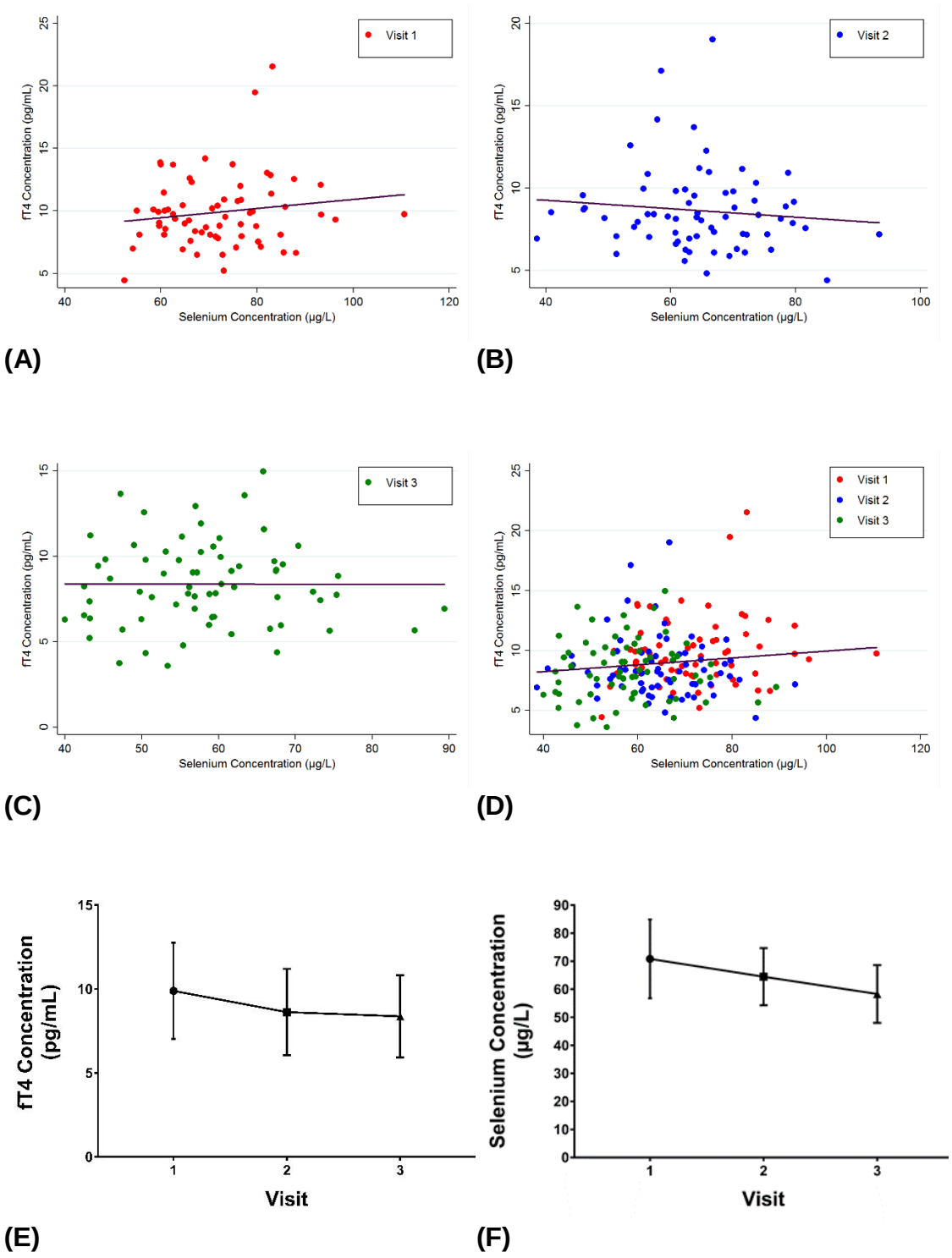


Figure 21: Correlations and moving averages for selenium and fT4. Graphs A-C show the relationship between selenium and fT4 across each visit (r_p= 0.15, -0.10 and -0.004, p>0.05 for all visits, respectively). Graph D shows the combined correlation for all visits throughout pregnancy (r_p=0.13, p=0.07). Graphs E and F show how mean fT4 and selenium concentrations change over time with 95% CI plotted for each time point.

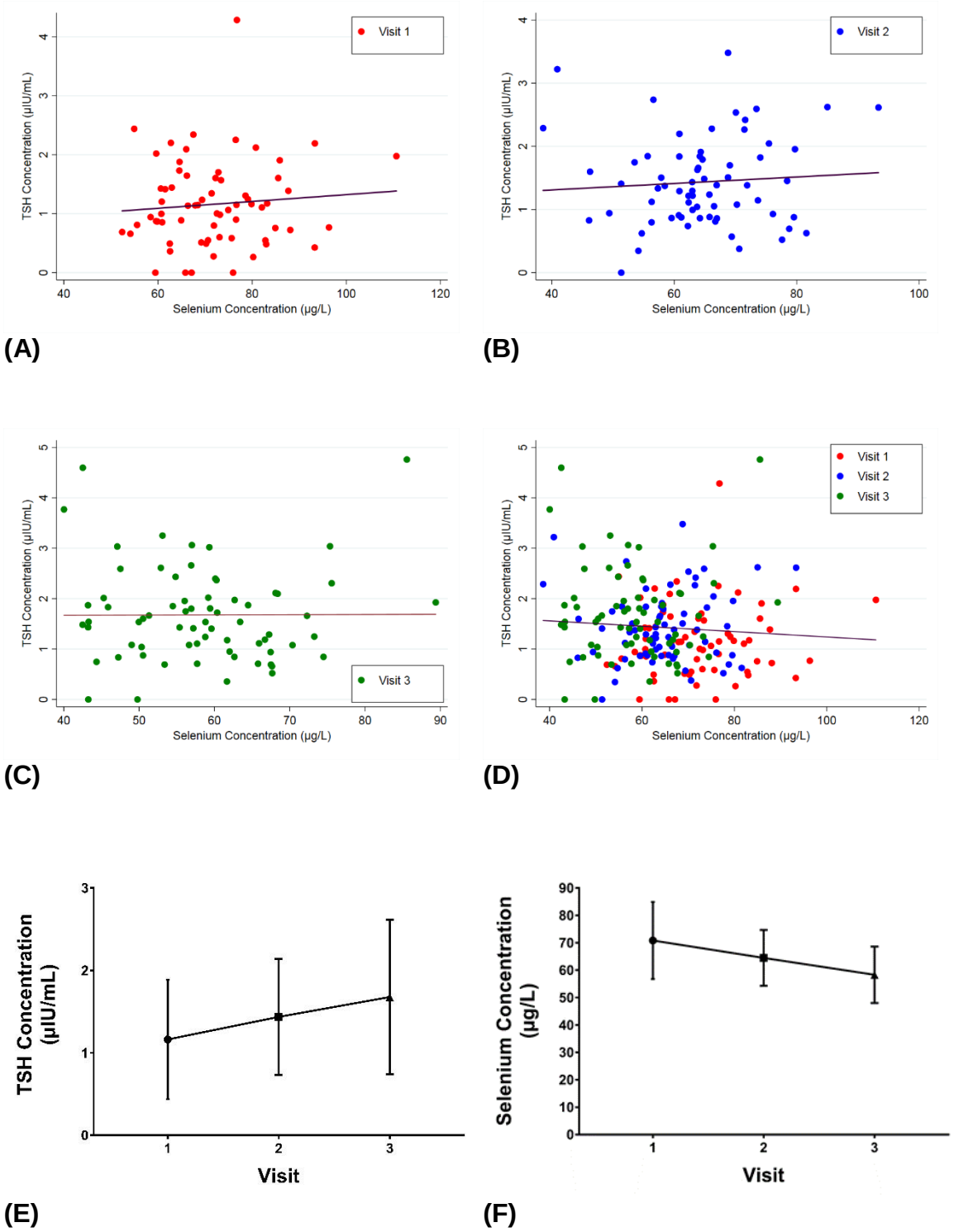


Figure 22: Correlations and moving averages for selenium and TSH. Graphs A-C show the relationship between selenium and TSH across each visit (r_p= 0.09, 0.07 and 0.004, p>0.05 for all visits, respectively). Graph D shows the combined correlation of all visits throughout pregnancy (r_p=-0.08, p=0.27). Graphs E and F show how mean TSH and selenium concentrations change over time with 95% CI plotted for each time point.

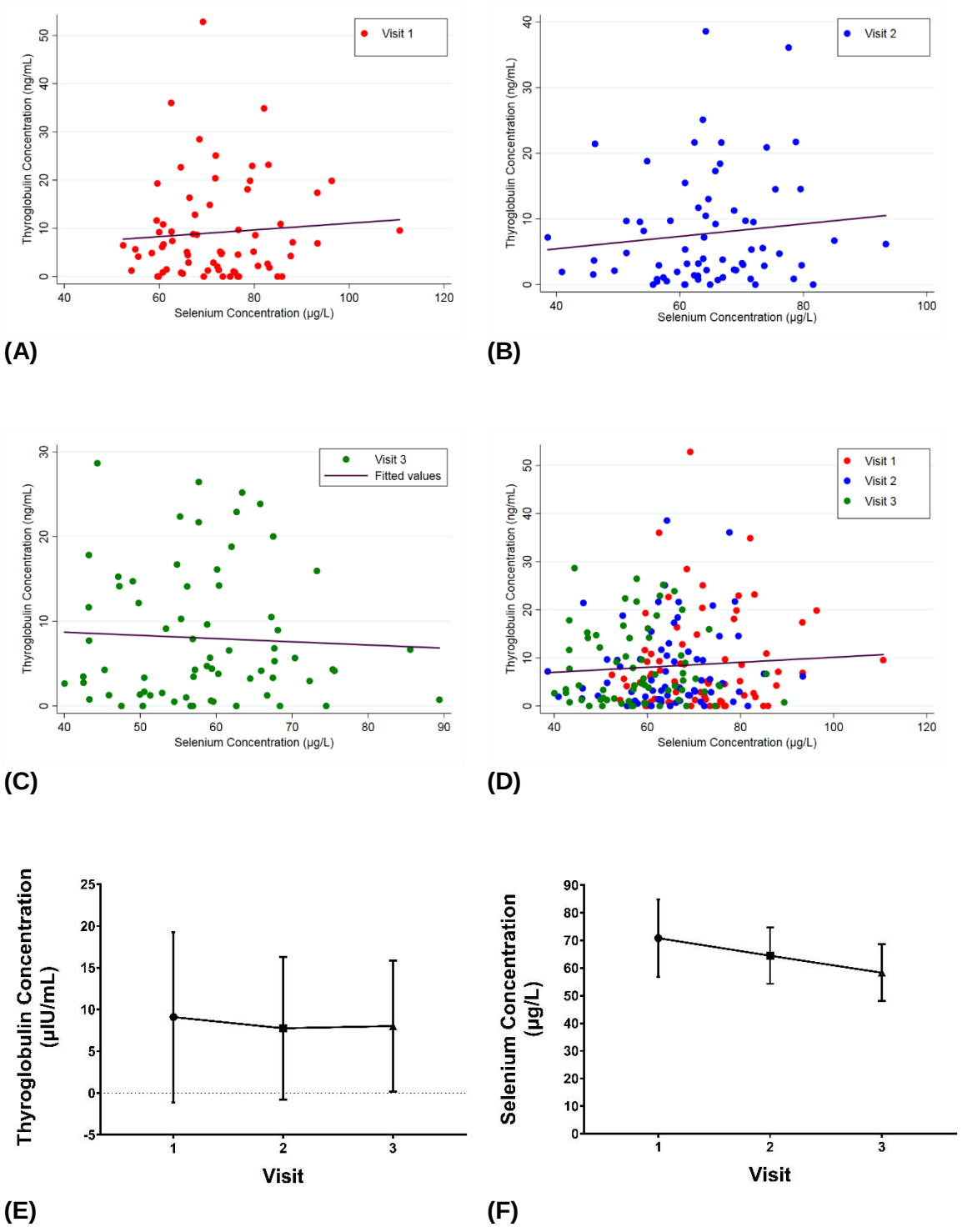


Figure 23: Correlations and moving averages for selenium and thyroglobulin. Graphs A-C show the relationship between selenium and thyroglobulin across each visit (r_p= 0.08, 0.11 and -0.05, p>0.05 for all visits, respectively). Graph D shows the combined correlation for all visits throughout pregnancy (r_p=0.07, p=0.32). Graphs E and F show how the mean concentrations of thyroglobulin and selenium changes over time with 95% CI plotted for each time point.

Thyroid Hormone	One way repeated measure ANOVA with Greenhouse-Geisser ([df_{time},$df_{residual}$]=F, p value)
fT3	F(2,138)=19.00, *p*<0.001
fT4	F(2,130)=13.08, *p*<0.001
TSH	F(2,133)=30.01, *p*<0.001
Thyroglobulin	F(2,133)=3.84, *p*= 0.03

Table 20: One-way repeated ANOVA for thyroid hormones. Results from the one-way repeated ANOVA analysis for all four thyroid hormones indicate that a statistically significant difference in concentrations was witnessed over gestation.

Visit	fT3:fT4 ratio
1	0.25
2	0.24
3	0.25

Table 21: fT3/fT4 ratios. fT3/fT4 ratios are displayed for each visit. There were no fluctuations in ratios as gestation progressed.

	Selenium (µg/L)			
	Unadjusted Estimates		Adjusted Estimates	
	β coefficient (CI 95%)	***p* value**	**β coefficient (CI 95%)**	***p* value**
GPx3 (ng/mL)	79 (60.0 to 99.0)	<0.001	68 (48.5 to 87.8)	<0.001
Selenoprotein P (ng/mL)	-24 (-32.5 to -16.2)	<0.001	-25 (-36.7 to -13.3)	<0.001
Iodine (µg/L)*	1.007 (1.00 to 1.02)	0.21	1.005 (0.99 to 1.02)	0.40
fT4 (pg/mL)	0.03 (-0.01 to 0.07)	0.12	0.03 (-0.02 to 0.08)	0.23
fT3 (pg/mL)	0.004 (-0.01 to 0.02)	0.55	0.008 (-0.01 to 0.02)	0.31
TSH (µIU/mL)	-0.005 (-0.02 to 0.01)	0.52	0.005 (-0.01 to 0.02)	0.52
Thyroglobulin (ng/mL)*	1.01(1.00 to 1.03)	0.15	1.01 (0.99 to 1.03)	0.30

Table 22: Repeat measure linear regression with selenium. Results show beta coefficients, 95% CI and *p*-values after repeat measure linear regression with GPx3, SEPP1, iodine, fT4, fT3, TSH and thyroglobulin. Adjusted estimates included age, smoking, alcohol consumption, BMI, ethnicity and vitamin supplementation for all variables. fT3, fT4, TSH and thyroglobulin was additionally adjusted for UIC. *Thyroglobulin and iodine were not normally distributed - values log transformed and regressed; results are presented as ratios.

3.6 Serum Selenium and Urinary Iodine Associations

Figure 24 depicts the relationships between urinary iodine and serum selenium concentrations at each study visit (Fig 24, A-C). Urinary iodine and serum selenium concentrations were used to conduct a Pearson's correlation. Using 207 observations across all three time points (Fig 24, D), the Pearson's coefficient was calculated as r_p= 0.11, CI 95% -0.03 to 0.25, *p*=0.11; indicating a non-significant small positive relationship between the two biomarkers. The moving average plot for iodine is shown in Figure 24, Graph E. At visits 1, 2, and 3, the mean concentration totalled 160.9, 145.1 and 169.8 µg/L, respectively. The variability in these concentrations is shown on the plots by the wide standard deviation, indicative of a small sample size. One-way repeated measure ANOVA with Greenhouse-Geisser correction was performed to indicate any significance between the differences of mean concentrations across visits. Results indicated that there was no significant difference in the means for iodine (F [2,136] =0.74, *p*=0.46). Table 22 shows the results from the unadjusted and adjusted repeat measure linear regressions with urinary iodine and serum selenium. As iodine was not normally distributed, data was log-transformed and beta coefficients displayed as a ratio. Both unadjusted and adjusted linear regression models show that selenium was not a statistically significant predictor of iodine concentration. A 1 µg increase in selenium resulted in a 0.7% increase in iodine concentration (β= 1.007, 95% CI 1.00 to 1.02, *p*=0.21,) without adjustment for confounding. A 0.5% increase in iodine concentration with 1 µg rise in selenium was calculated with adjustments for age, smoking, BMI, alcohol consumption, supplementation usage and ethnicity (β= 1.005, 95% CI 0.99 to 1.02, *p*=0.40).

To explore potential synergy (statistical interaction) between selenium and iodine levels and their relationships with thyroid hormones, participants were divided into two groups for both selenium and iodine. This was determined by calculating whether participants' values were above or below the median level. Those in the 'high' groups had concentrations above the median, those participants in the 'low' group had concentrations below the median. This gave rise to four groups: 'high' in both selenium and iodine, 'low' in both selenium and iodine, 'high' iodine but 'low' selenium and 'high' selenium but 'low' iodine. Median concentrations for GPx3, SEPP1, fT3, fT4, TSH and thyroglobulin were assessed based on these differing scenarios.

Results are displayed in Table 23. Whilst most biomarkers showed little variation between the groups, there was some evidence that GPx3 was particularly low in mothers who had 'low' selenium and 'low' iodine compared to those mothers that had 'high' selenium and 'high' iodine (5800 µg/L vs 7400 µg/L respectively). An opposite pattern showing increasing thyroglobulin between the same two groups (6.9 ng/mL vs 4.5 ng/mL) was also observed.

		Selenium			
		Low		High	
Iodine	Low	GPx3[1]	5800	GPx3[1]	7500
		SEPP1[1]	4000	SEPP1[1]	3600
		fT3[2]	2.1	fT3[2]	2.2
		fT4[2]	8.3	fT4[2]	8.3
		TSH[3]	1.4	TSH[3]	1.1
		Thyroglobulin[1]	6.9	Thyroglobulin[1]	6.2
	High	GPx3[1]	6700	GPx3[1]	7400
		SEPP1[1]	4200	SEPP1[1]	3800
		fT3[2]	2.2	fT3[2]	2.1
		fT4[2]	9.0	fT4[2]	8.8
		TSH[3]	1.2	TSH[3]	1.2
		Thyroglobulin[1]	4.1	Thyroglobulin[1]	4.5

Table 23: Iodine and selenium interactions. Table 22 shows the median values of each biomarker as per the high/low iodine and selenium groups. [1](ng/mL), [2](pg/mL), [3](µIU/mL)

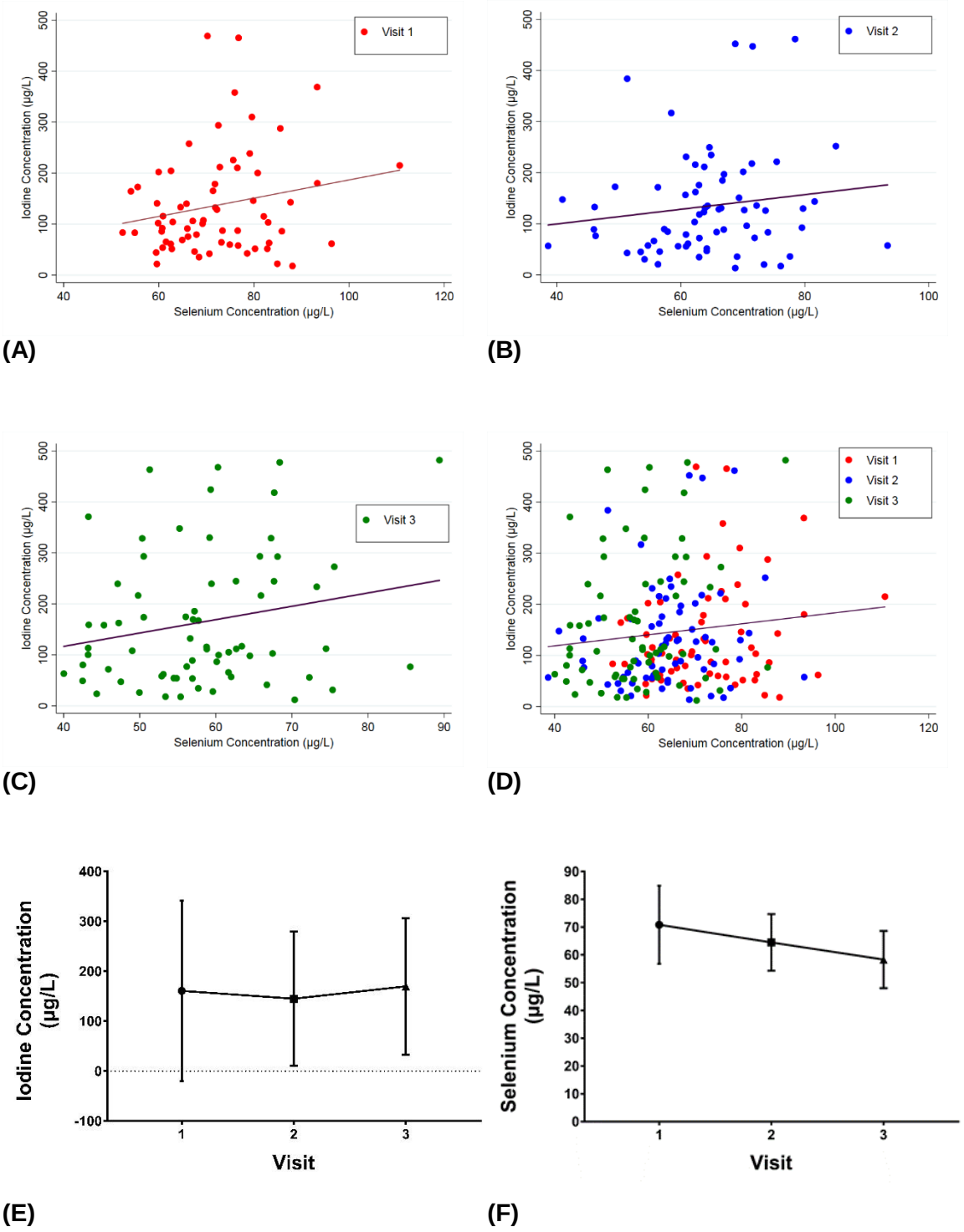


Figure 24: Correlations and moving averages for selenium and iodine. Graphs A-C show the relationship between selenium and iodine across each visit (r_p= 0.20, 0.14 and 0.20, p>0.05 for all visits, respectively). Graph D shows the combined correlation for all visits throughout pregnancy (r_p=0.11, p=0.10). Graphs E and F show how the mean concentrations of iodine and selenium concentrations change over time with 95% CI plotted for each time point.

3.7 Dietary Selenium and Serum Selenium

The relationship between dietary selenium intake and serum selenium for each visit is depicted by Figure 25. Dietary selenium intake was quantified for each individual at each visit using the myfood24 tool (see section 2.1.4). Pearson's correlation using dietary selenium values and serum selenium was used to generate a Pearson's coefficient at visit 1 (Fig 25, A - r_p=0.14, 95% CI -0.10 to 0.36, *p*=0.25). Visit 2 (Fig 25, B) gave a result of r_p=-0.01, 95% CI -0.25 to 0.22, *p*=0.92 and visit 3 (Fig 25, C) produced a coefficient of r_p=0.11, 95% CI -0.1 to 0.33, *p*=0.37. A combined coefficient covering all visits (Fig 25, D) was r_p=0.03, 95% CI -0.10 to 0.2, *p*=0.62. The results show that during visit 3, there was a very weak, positive correlation between serum selenium and dietary selenium. Correlation analysis with serum selenium at visits 1 and 2 and with all visits combined (Graph D) showed no relationship. None of the results were statistically significant.

Repeat Measure linear regression was performed to investigate whether dietary selenium was a significant predictor of serum selenium status. Unadjusted results (β=0.02, 95% CI -0.06 to 0.93, *p*=0.64) and results adjusted for age, ethnicity, smoking, alcohol consumption, BMI and vitamin supplementation (β=-0.01, 95% CI -0.10 to 0.071, *p*=0.76) showed that dietary selenium was not a significant predictor of serum selenium status. For every 1 µg of dietary selenium consumed, there was a 0.02 µg/L increase in serum selenium (unadjusted regression model, not statistically significant). However, when adjusted for confounding, results displayed a slight decrease in serum selenium levels (-0.01 µg/L decrease in serum selenium for every 1 µg of dietary selenium consumed, not statistically significant).

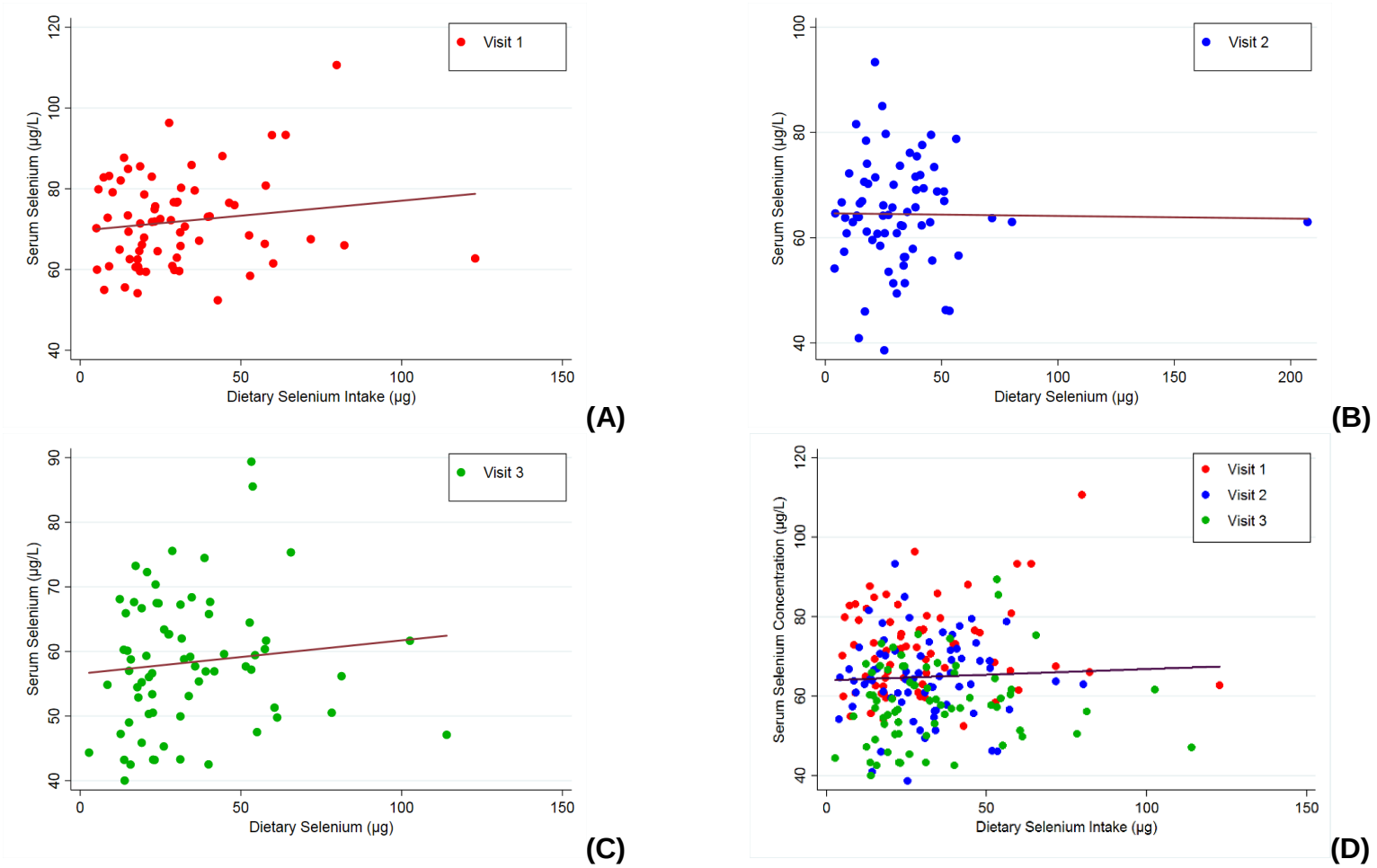


Figure 25: Relationship between dietary selenium intake and serum selenium concentration. Graphs A-C show the relationship between dietary selenium and serum selenium across each visit. Graph D shows the combined correlation for all visits throughout pregnancy

3.8 Serum Selenium, Glutathione Peroxidase 3 and Selenoprotein P and their Associations with Birthweight

Figure 26 depicts the relationships between serum selenium levels and birthweight for the 70 participants at each visit (Fig 26, A-C) and combined for all visits (Fig 26, D). Using the normally distributed data, a Pearson's correlation was performed. The Pearson's coefficient for visit one was calculated as r_p=0.24, 95% CI -0.010 to 0.463, *p*=0.06, with visit two and visit three having coefficients of r_p=0.008, 95% CI -0.240 to 0.255, *p*=0.949 and of r_p=-0.057, 95% CI -0.301 to 0.193, *p*=0.656, respectively. At visit one, there was a weak positive relationship with selenium and birthweight which was slightly above the statistical significance threshold (*p*=0.06). The remaining two visits throughout pregnancy showed no correlation. The overall coefficient for all combined visits was of r_p=0.06, CI 95% -0.086 to 0.200, *p*=0.43 (Fig 26, D) signifying no overall association between birthweight and selenium concentration throughout the entirety of pregnancy.

Table 24 shows the results from the unadjusted and adjusted repeat measure linear regressions between selenium and birthweight at each visit. Serum selenium was found to be a significant predictor of birthweight at visit one. For every 1 µg increase in selenium concentration during the first visit, birthweight significantly increased by 15 g; (results adjusted for age, ethnicity, smoking, alcohol consumption, UIC, BMI and vitamin supplementation, β=15, CI 95% 2 to 30, *p*=0.02). However, at visits two and three, selenium was not a significant predictor of birthweight (β= -4, 95% CI -20 to 10, *p*=0.67 and β=2, 95% CI -10 to 20, *p*=0.77 respectively). GPx3 follows a similar trend to that of selenium (Table 25). Unadjusted and adjusted repeat measure linear regressions showed that GPx3 was a significant predictor of birthweight at the first visit; a 0.11g increase in birthweight was witnessed for each 1 ng increase in GPx3 (β=0.11, CI 95% 0.009 to 0.2, *p*=0.032). However, no such associations were found during the later trimesters (*p*=0.776 and *p*=0.263 for visits two and three respectively). SEPP1 and its associations with birthweight can be seen in Table 26. Unlike GPx3 and serum selenium, SEPP1 has a significant inverse association with birthweight at visits one and two (adjusted results β= -0.18, 95% CI -0.31 to -0.05, *p*=0.008 and β= -0.25, 95% CI -0.40 to -0.09, *p*=0.002, respectively). For every 1 ng increase in SEPP1, there was a decrease in birth weight by 0.18 and 0.25 g for visits one and two, respectively. SEPP1 was found not to be a significant predictor of birthweight during the third trimester (*p*=0.385).

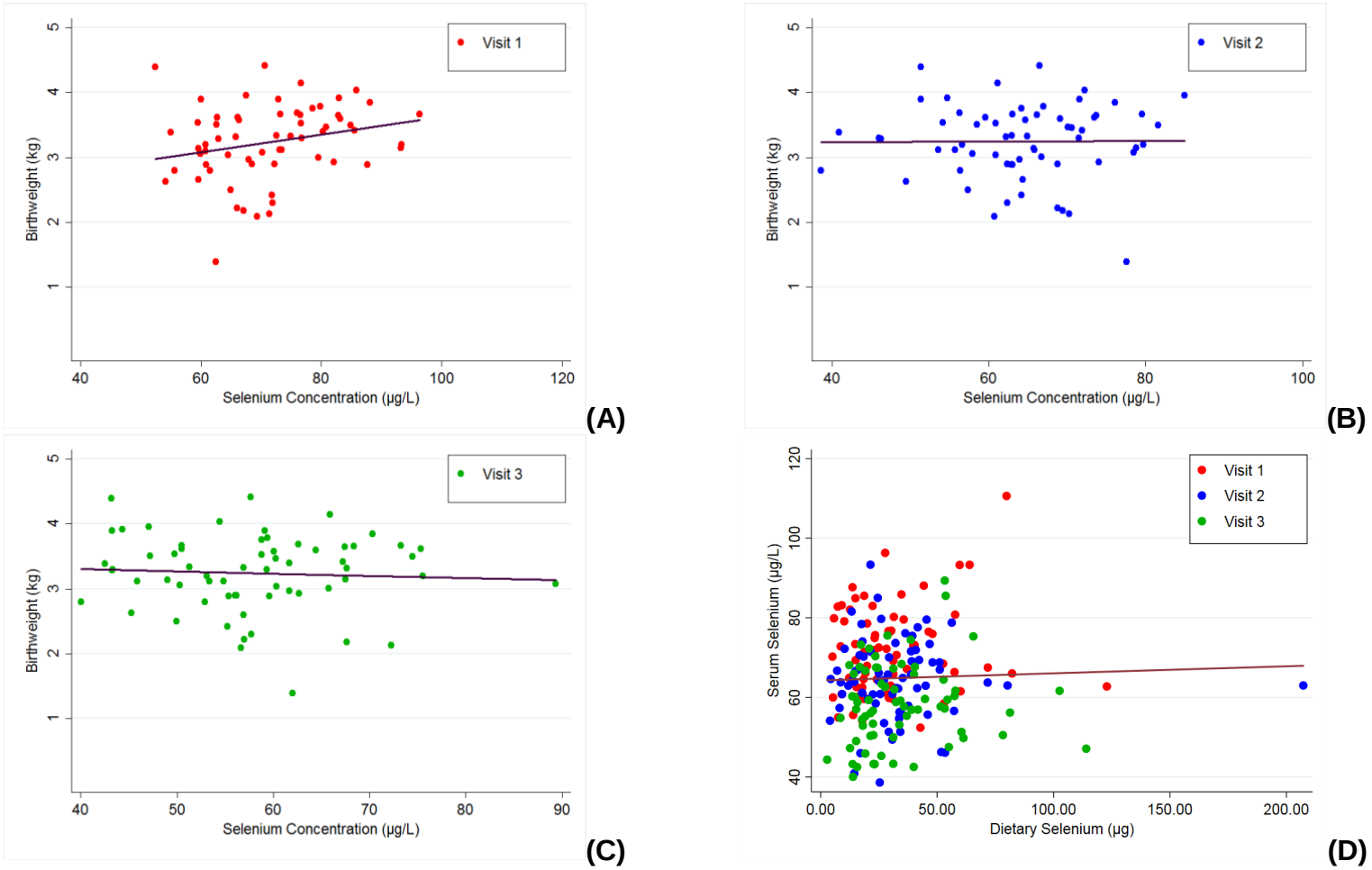


Figure 26: Relationship between selenium concentration and birthweight. Graphs A-C show the relationship between serum selenium and birthweight across each visit. Graph D shows the combined correlation for all visits throughout pregnancy.

	Selenium (µg/L)			
	Unadjusted Estimates		Adjusted Estimates	
Birthweight (g)	B coefficient (CI 95%)	*p* value	B coefficient (CI 95%)	*p* value
Visit 1	14 (0.4 to 30)	0.04*	15 (2 to 30)	0.02*
Visit 2	0.4 (-10 to 20)	0.95	-4 (-20 to 10)	0.67
Visit 3	-3 (-20 to 10)	0.60	2 (-10 to 20)	0.77
All Visits	2 (-5 to 10)	0.45	4 (-5 to 10)	0.34

Table 24: Repeat measure linear regression between selenium and birthweight. Beta coefficients, 95% CI and p-values generated from repeat measure linear regression between selenium and birthweight. Adjusted estimates included age, smoking, alcohol consumption, BMI, ethnicity, UIC and vitamin supplementation.* $p<0.05$, statistical significance indicated.

	GPx3 (ng/mL)			
	Unadjusted Estimates		Adjusted Estimates	
Birthweight (g)	B coefficient (CI 95%)	*p* value	B coefficient (CI 95%)	*p* value
Visit 1	0.1 (0.01 to 0.18)	0.019*	0.11 (0.009 to 0.2)	0.032*
Visit 2	0.01 (-0.073 to 0.10)	0.745	0.02 (-0.10 to 0.14)	0.776
Visit 3	-0.06 (-0.16 to 0.04)	0.269	-0.05 (-0.15 to 0.04)	0.263
All Visits	0.01 (-0.06 to 0.09)	0.702	0.02 (-0.07 to 0.10)	0.420

Table 25: Repeat measure linear regression between GPx3 and birthweight. Beta coefficients, 95% CI and *p*-values after repeat measure linear regression between GPx3 and birthweight. Adjusted estimates included age, smoking, alcohol consumption, BMI, ethnicity, UIC and vitamin supplementation. * p<0.05, statistical significance indicated.

	SEPP1 (ng/mL)			
	Unadjusted Estimates		Adjusted Estimates	
Birthweight (g)	B coefficient (CI 95%)	*p* value	B coefficient (CI 95%)	*p* value
Visit 1	-0.14 (-0.28 to 0.02)	0.087*	-0.18 (-0.31 to -0.05)	0.008*
Visit 2	-0.24 (-0.38 to -0.09)	0.002*	-0.25 (-0.40 to -0.09)	0.002*
Visit 3	-0.11 (-0.30 to 0.09)	0.274	-0.09 (-0.28 to 0.11)	0.385
All Visits	-0.14 (-0.27 to -0.004)	0.043*	-0.18 (-0.30 to -0.06)	0.005*

Table 26: Repeat measure linear regression between SEPP1 and birthweight. Beta coefficients, 95% CI and *p*-values after repeat measure linear regression between SEPP1 and birthweight. Adjusted estimates included age, smoking, alcohol consumption, BMI, ethnicity, UIC and vitamin supplementation. * p<0.05, statistical significance indicated.

Discussion

4.1 Selenium, Glutathione Peroxidase 3 and Selenoprotein P

Selenium adequacy at population level is based on the concentration required to maximise GPx3 output. This threshold is currently set at 90-100 µg/L as proposed by Duffield et al. (1999) and Thomson et al. (1977) whilst assessing New Zealand selenium status in the general population. As per this guidance, the population of women assessed in this study cohort were found to have selenium levels considerably lower than these proposed optimal concentrations (see Table 18). Serum selenium status in this study was similar to a Spanish study which also quantified selenium status across all three trimesters of pregnancy (Navarro et al., 1996). Mean concentrations in the Spanish study indicated that selenium was sub-optimal throughout gestation, however it remained fairly consistent across the trimesters (64.6, 66.6, 61.6 µg/L respectively). Furthermore, a Polish study also showed that selenium status fell linearly as gestation progressed, reaching its lowest concentration just before birth (35.7 µg/L between 35-40 weeks gestation) (Zachara et al., 1993).

The selenium inadequacy seen in this study could be due to a number of factors. Firstly, pregnant women may not be obtaining the required dietary selenium intake to support both maternal demands and placental transfer of selenium to the foetus for growth and development (Mistry et al., 2014; Zimmermann and Kohrle, 2002). European soils, including the UKs, are known to be lower in selenium concentration compared to other continents such as the Americas. This in turn can give rise to dietary selenium insufficiency. Selenium enters the food chain through plant amino acid metabolism, therefore low soil concentration means less selenium uptake by mammals and a reduced amount of the micronutrient in certain foods (Schomburg and Kohrle, 2008). This low selenium intake aligns with the latest NDNS survey where 47% of women at child bearing age were below the lower reference nutrient intake for selenium (there are no measurements for women during pregnancy) (Public Health England, 2018). Trading regulations can also have an impact on selenium status of a population. For example, the reduction of flour imports from the USA after the UK joined the EU saw the UK turn to local flour varieties. The

implication of this was a sharp fall of selenium content in bread and inherently, the general population (Rayman, 1997).

The decreasing levels of serum selenium throughout gestation could also be the result of plasma volume expansion (PVE). During pregnancy, PVE is necessary to facilitate the increased circulatory needs of the placenta and maternal organs. With on average, an increase by ~45% throughout gestation, PVE could be diluting serum selenium concentration, leading to these low levels (Faupel-Badger et al., 2007).

As expected, the correlations shown in Figure 17 and the results of the regression analysis show that selenium is a significant predictor of GPx3 expression throughout the course of pregnancy. Similar results have been shown in a study by Zachara et al. (1993) who found plasma selenium levels in pregnant Polish women correlated positively with plasma GPx activity. This adds to the body of research showing that increased selenium concentrations through adequate selenium intake results in increased GPx3 synthesis. Sec, the core selenium constituent of selenoproteins, is reliant upon sufficient selenium availability for optimal protein expression and is sensitive to decreased selenium intake (Duntas and Benvenga, 2015). Low levels of GPx3 throughout pregnancy could also indicate an increased need for anti-oxidative protection during growth and development. An increase in oxygen requirements for both mother and child subsequently increases production of ROS. This is due to an increase in metabolic rate and increased oxygen consumption (Soma-Pillay et al., 2016) Owing to its antioxidant properties, high concentrations of GPx3 may be needed to ensure adequate protection (Pieczynska and Grajeta, 2015).

This study unexpectedly showed that serum selenium had an inverse association with SEPP1 (as seen in Figure 18). This is in contrast with studies such as Santos et al. (2017) who found selenium was a positive predictor of SEPP1 status. With 10 selenocysteine residues per SEPP1 molecule and the fact that SEPP1 is reflective of ~50% of plasma selenium (Papp et al., 2007), it was anticipated that the higher levels of selenium needed for selenocysteine generation would result in higher levels of SEPP1, however the results of this study did not reflect this. The increase in median SEPP1 concentration throughout gestation was also unexpected. Whilst literature is lacking for trimester specific concentrations of SEPP1 and its potential consequences during pregnancy, Burk et al. (2013) found in mice models that SEPP1 concentration decreased rather than increased through the later stages of gestation due to receptor mediated uptake of SEPP1 to the developing foetus. Therefore, within this study a potential explanation for the increase in SEPP1 could be that the low selenium environment witnessed in this cohort of women has prompted SEPP1

facilitation to compensate for this deficit. As the main transporter and distributor of selenium, the increased demands during pregnancy could be causing selenium homeostasis imbalance (Ventura et al., 2017).

It is also been shown that foetal stores of selenium accumulate in the liver, kidneys and heart towards the end of the third trimester. A study in Poland showed the selenium concentrations in all three of these organs were higher in deceased full-term infants compared to deceased premature foetuses (Zachara et al., 2001). It has therefore been suggested that this could be the result of an active transport system of selenium via SEPP1 across the placenta during the latter stages of pregnancy (Kasik and Rice, 1995).

Selenoproteins are organised and expressed in a specific hierarchy in periods of limited selenium status (Schomburg and Schweizer, 2009). SEPP1 is relatively high in the hierarchy, making it one of the most stable selenoproteins under selenium deficient states. This allows the concentrations of this protein to remain conserved in critical organs such as the brain and thyroid (Solovyev et al., 2018; Hill et al., 2003). In comparison, GPx3 is much lower in the hierarchical system, resulting in falling concentrations of this protein in a selenium deficient state (Brigelius-Flohe and Maiorino, 2013; Mostert, 2000). The results seen from this study looks to support this theory. SEPP1 concentration appears not to be affected, increasing throughout pregnancy whereas GPx3 is more sensitive to selenium status, resulting in decreased concentrations as serum selenium falls throughout gestation. This is likely to be the result of premature termination of GPx3 protein synthesis or a decreased concentration of GPx3 mRNA expression (Wingler et al., 1999).

It is apparent from this study that the selenium concentration in this UK cohort of women is not sufficient to sustain GPx3 concentration. At present, there is no UK specific guidance for pregnant women regarding the levels of selenium required to sustain a healthy pregnancy. Given that selenium is an essential micronutrient for selenoprotein expression and thyroid hormone activation in foetal development (Chan et al., 2003), research is urgently required to provide reliable estimates that will optimise selenium and selenoproteins during this critical time. In addition, the most appropriate biomarker to determine selenium adequacy is not well established i.e. SACN guidelines refers to the concentration of selenium required for maximal GPx expression whereas the European Food Safety Authority use maximal SEPP1 expression in order to derive dietary intakes (EFSA Panel on Dietetic Products and Allergies, 2014; SACN, 2013). This inconsistency warrants further study to establish universal definitions on how to assess selenium status. Whilst dietary intake of

selenium will vary by country as geochemical conditions change, it is only reasonable that the same biomarker should be used in comparisons for consistency. This will facilitate a greater understanding within the field.

4.2 Iodine and Thyroid Hormones

A median urinary iodine concentration of 150-249 µg/L defines iodine sufficiency during pregnancy as per the WHO guidelines. Where concentrations fall below the 150 µg/L threshold, pregnant populations would be deemed as iodine insufficient, indicative of suboptimal iodine intake (World Health Organisation, 2007). As per these limits, the cohort of women within this study would be classified as iodine deficient across the entire duration of pregnancy (103.4, 120.8 and 112.1 µg/L respectively). Results found here reflect the current body of existing research. A UK based longitudinal study performed by Furmidge-Owen et al. (2014) witnessed extremely low iodine levels across all three trimesters of pregnancy (42, 52 and 69.4 µg/L respectively). A Spanish study also found suboptimal iodine levels throughout the first two trimesters of pregnancy. 79.8% (median UIC: 88.5 µg/L) and 54.4% (median UIC: 40 µg/L) of women at trimesters one and two respectively, had an UIC lower than the 150 µg/L adequacy limit (Aguayo et al., 2013). Bath et al. (2014) found that the median urinary iodine concentration (UIC) in a population of 100 women in the South East of England during the first trimester was 85.3 µg/L and Snart et al. (2019) found women across three locations in the UK (Leeds, London and Manchester) were iodine insufficient in the second trimester of pregnancy (median UIC levels 116, 130 and 139 µg/L for each location respectively). However, a Bangladeshi study by Rydbeck et al. (2013) found that median UIC concentrations from a population of 271 pregnant women rose throughout gestation and had adequate UIC (241, 268, 296 and 300 µg/L at 8, 14, 19 and 30 weeks respectively). In a geographical location known for optimal iodine intakes within the general population, it is no surprise to see adequate UIC levels in the Bangladeshi cohort of women. This will most likely be the result of a mandatory USI programme implemented within this area, providing adequate iodine intake on a population level (Yusuf et al., 2008). However, the results obtained from the Spanish and UK studies represents the ongoing iodine insufficiency experienced throughout the majority of Europe (Zimmermann and Boelaert, 2015). Geochemistry, combined with a lack of mandatory USI programmes in these European areas gives rise to insufficient iodine intake resulting in low UIC status (Zimmermann, 2009b). The lowest UIC in the current study was reported at the first visit (103.4 µg/L). During this early period of

gestation, thyroid hormone receptors present in the foetal brain ensure normal foetal myelination via maternal T4 placental transfer (Gernand et al., 2016). Low UIC status witnessed in this study could therefore be increasing the risk of IDD's such as neurological cretinism due to an increased risk of potential impairment of sufficient thyroid hormone metabolism (Zimmermann, 2011).

Basic ethnicity analysis showed a significant increase in mean iodine concentration for Pakistani women compared to those of White-British ethnicity (Table 17). The results from an Australian study by Hamrosi et al. (2005) mirrored this study's findings; pregnant Caucasian groups had a lower overall UIC concentration compared to Vietnamese and Indian/Sri Lankan women. There is known to be a number of differing predispositions within ethnic groups such as potential genetic variabilities, socioeconomic status, dietary intake and general cultural differences, all of which could have had an impact on UIC status. Further exploratory subgroup analysis with a larger sample size would be beneficial as the small numbers in each group (as seen within this study) leads to difficulty interpreting results due to the low statistical power.

There were no major fluctuations in thyroid hormone concentrations across pregnancy (Table 18). A Dutch study showed a very similar trend for serum TSH and fT4 levels in comparison with our project. With consistent small increases in TSH (1.23-1.51μIU/mL) and small decreases in fT4 (12.4-10.3pg/mL), results were indicative of a population with normal thyroid function throughout the entirety of pregnancy (Monen et al., 2015). During normal pregnancy, fT3, fT4 and TSH concentrations can be attributed to the stimulatory effect of the pregnancy hormone hCG. A fall in TSH concentration of up to 50% and a rise in fT3 and fT4 have been witnessed, peaking during the first trimester at around 10-12 weeks gestation. As gestation progresses, TSH starts to rise and fT3/fT4 decreases, however the precise reasons for this is still unclear (Lazarus, 2011). Reference limits have also been recommended for TSH and fT4 in the absence of laboratory specific reference ranges. According to guidelines set by the European Thyroid Association and The Endocrine Society, TSH values are within a normal range if concentrations are below 2.5μIU/mL in trimester 1 and 3μIU/mL in trimesters 2 and 3 (Lazarus et al., 2014; De Groot et al., 2012). The American Thyroid Association have recently updated their guidelines to reflect that the upper limit should not surpass 4μIU/mL (Alexander et al., 2017). Whilst we have no baseline data to compare the drop in TSH levels from pre-pregnancy to first trimester, if we were to apply the results to the criteria provided, women in this cohort appear to have normal thyroid hormone concentrations which

is indicative of a normal pregnancy and a subsequent low risk of developing subclinical or overt hypothyroidism.

Reference ranges for thyroid hormone function tests differ throughout gestation compared to the non-pregnant population (Lazarus, 2011). Profound physiological changes in the thyroid as a result of increased demands from the foetus causes increases in thyroid hormones to meet maternal and foetal requirements (Stricker et al., 2007). Therefore, it is imperative that trimester specific reference ranges during pregnancy must be used during this period. At present, pregnancy criteria for thyroid hormone concentrations tends to be centre specific between countries/populations; there is no global guidance on reference ranges due to a number of influencing factors such as iodine status, ethnicity, BMI, age and laboratory methodologies used (Moon et al., 2015; Medici et al., 2015). This is understandable when studies such as Li et al. (2014) demonstrate the pitfalls of using of global TSH criteria during pregnancy. 4800 Chinese women had TSH assessed against both centre specific criteria (upper limit TSH>4.87mIU/L) and the American Thyroid Association criteria (upper limit TSH>2.5mIU/L at time of analysis). It was found that during 4-12 weeks gestation, 27.8% of the women would be classed as at risk of subclinical hyperthyroidism by American Thyroid Association criteria compared to just 4% of women when using centre specific reference ranges. This proves the variation and influencing factors between countries. However, the current WHO UIC criteria for pregnancy is a globally accepted measure of iodine status irrespective of the current iodine status of a country, ethnicity/BMI of the population and the laboratory methodologies used. When the influencing factors are the same for UIC reference ranges, it could be asked why there are no globally accepted gestational thyroid hormone limits? It could be beneficial to implement a standardised laboratory methodology for the determination of thyroid hormone reference ranges to eliminate one of these factors of variability. For example, there is controversy surrounding which method is suitable for this quantification. Some literature states that immunoassay is a sufficient measure whereas others claim LC/MS is the ‘gold standard’ (Lazarus et al., 2014). The WHO EQUIP programme has aimed to standardise UIC determination. A similar approach could well be needed to define more accurate pregnancy specific thyroid hormone reference ranges on a more global scale.

It would have been presumed that iodine and associated thyroid hormones would be associated in some capacity due to iodine being a direct regulator of thyroid hormone expression. However, this study did not find evidence for this. There were weak/no associations between fT3, fT4 and TSH with UIC throughout pregnancy (Table 18). A number of studies have found similar results. A Spanish study by Aguayo et al. (2013) and a French study by Luton et al. (2011) found little evidence of any associations between UIC and these corresponding thyroid hormones. The results from this study could be due to UIC analysis not being a good enough measure of iodine status within this small cohort. Hydration levels affect smaller sample sizes more profoundly in comparison to studies with larger sample sizes, leading to common misinterpretations (Zimmermann, 2009a). Also, iodine stores may have been sufficient prior to pregnancy. It is thought that adequate iodine concentrations before gestation are able to maintain both maternal and foetal hormone requirements (Yarrington and Pearce, 2011). However, without baseline data, this is hard to deduce. Although there are no associations between UIC and fT3, fT4 and TSH, there is a suggestion that thyroglobulin and UIC may be inversely associated, though this observation did not reach statistical significance (p=0.06). Whilst the result did not display a significant result, the overall trend shown agrees with Bath et al. (2017) who also found an inverse association of thyroglobulin with UIC in the UK based SPRINT cohort. Thyroglobulin is thought to be a very sensitive marker of iodine deficiency – a raised concentration is indicative of thyroid enlargement and stress resulting in the release of this protein into the blood circulation (Ma and Skeaff, 2014). The results from this study reflect this observation as thyroglobulin concentrations tends to be higher in individuals with a lower UIC status.

This study design relied on spot urine collection to measure UIC. As briefly mentioned, spot urines are more effective with bigger sample sizes as variable hydration levels between individuals are levelled out within larger populations (Zimmermann, 2009). For this study, the low sample size (70 participants) means that spot UIC levels may be influenced by a number of factors including hydration levels and the diurnal variation of iodine (Alexander et al., 2017). This can lead to common UIC misinterpretations. To overcome these limitations, the use of 24-hour urine samples would have been the preferred method of collection (Zimmermann, 2009), however, the impracticalities of doing so meant that spot urine analysis was the only feasible way of determining UIC in this cohort.

4.3 Selenium, Iodine and Thyroidal Interactions

Sufficient selenium and iodine intakes are imperative for normal thyroid hormone synthesis and metabolism (Zimmermann and Kohrle, 2002). This is the only UK based cohort study that has assessed both selenium intake and its potential impact on iodine and thyroid hormones throughout gestation. Results from the regression analysis after adjustments for confounding showed that there were no significant associations between selenium and iodine (p=0.40), selenium and fT4 (p=0.20), selenium and fT3 (p=0.34), selenium and TSH (p=0.50) and selenium and thyroglobulin (p=0.31). Due to a lack of research investigating these associations throughout pregnancy, comparisons have been undertaken in non-pregnant populations. Our findings reflect the results found in two New Zealand studies. Thomson et al. (2007a) found no correlation or association with selenium and thyroid status (fT4, fT3 and TSH) in both male and female adults, however, positive correlations were witnessed between urinary selenium and urinary iodine (p<0.001). In the second study, Thomson et al. (2009) found that in 100 subjects aged 60-80 year olds, there was no interactions between mild iodine deficiency and low selenium status.

The small non-significant positive correlation between serum selenium and urinary iodine at all visits (Figure 24) could potentially be attributed to dietary and supplementary intake. Some food items such as fish are rich sources of both selenium and iodine. In addition, for those women taking supplements containing both selenium and iodine, results would be expected to be slightly correlated as women would be consuming the required dietary intake of both micronutrients. In contrast, the lack of a significant association could be due to the specimen collection methods. Firstly, differences in matrices could have an effect on results (serum selenium vs urinary iodine). Spot urines collected for the project are very susceptible to large amounts of variation due to reasons as described in section 4.2. This could have had an influential effect when calculating accurate iodine measurements. In addition, selenium and iodine are utilised in completely different metabolic pathways. The only known biological role for iodine is T3 and T4 synthesis (Arthur et al., 1999) and selenoprotein production only requires selenium specifically for Sec production (Papp et al., 2007). There is no further requirement for either of these trace elements other than for their respective protein pathways i.e. T3 and T4 do not require selenium for synthesis, selenoproteins do not require iodine.

As the key mediator in thyroid hormone metabolism, DIO require sufficient selenium for Sec production. This amino acid is key for normal DIO1 and DIO2 functionality, catalysing the 5' outer ring to produce T3 from T4 (Labunskyy et al., 2014). In periods of selenium deficiency as seen in this study, it would have been expected to potentially see an inverse association with selenium and T4 due to potential impairment of DIO expression (lack of DIO, reduced conversion of T4 to T3, higher T4 concentrations). As defined by Thomson (2004), optimal deiodinase expression occurs when serum selenium concentrations reach ~65 µg/L. This threshold was met during the first visit only; serum selenium falls short of this limit during the latter two time points. However, overall the results seem to suggest serum selenium was sufficient for DIO expression throughout gestation. Firstly, TSH and T4 concentrations remained within their expected normal limits throughout pregnancy. If there was any deiodinase impairment, we may have seen a sharp rise in concentration of both of these thyroid hormones. The T3/T4 ratio assessment can be implied as a good indicator of DIO activity (Zimmermann and Kohrle, 2002). As seen in Table 21, the consistent fT3/fT4 ratios for all visits across pregnancy indicates consistent conversion from fT4 to the active thyroid hormone fT3, hence normal DIO activity (Olivieri et al., 1996; Thomson et al., 2009). Thirdly, as selenium concentration is at its lowest within the last trimester, there is no association between selenium and fT4 at this time point – an inverse association would have been expected should there had been a decline in DIO expression. Lastly, it may be the case that the 65 µg/L threshold set by Thomson (2004) may not be absolute. The selenoprotein hierarchy ranks the DIO family of selenoproteins highly. This means in periods of limiting selenium supply, these selenoproteins are continually expressed (Bermano et al., 1995). In this study, it could be the case that the selenium concentration is sufficient for DIO expression and is prioritised over other selenoproteins such as the more selenium sensitive GPx3 (as seen by the falling median concentration of GPx3 as gestation progresses) to ensure that thyroid hormone metabolism is maintained.

It would have been hugely beneficial to measure the concentration of maternal DIO (DIO1, DIO2 and DIO3) in this study due to their pivotal role in thyroid hormone metabolism. This would have enabled us to investigate how DIO concentrations change (if at all) throughout pregnancy and to determine if lowering selenium concentration had any impact on the DIO expression. This analysis would be a key priority in any future studies.

As participants in this study were only mildly iodine deficient and selenium insufficient, further research and studies are warranted to establish if and how mild deficiencies have the potential to effect the thyroid axis. It is well known that in periods of selenium deficiency, thyroidal selenium and selenoprotein regulation tends to be unaffected due to the selenoprotein hierarchical system (Bermano et al., 1995). It is therefore more likely that interactions between the two micronutrients may only have a profound effect when exhibiting severe deficiencies in iodine and selenium i.e. as seen in the manifestation of myxoedematous cretinism.

4.4 Selenium and Dietary Intake

This is the first UK study to assess dietary selenium intake and its impact on selenium concentration throughout gestation. Results from correlation and regression analyses found that dietary selenium assessment was not a significant predictor of serum selenium status. Previous research investigating this association has been conflicting. A study by Thomson et al. (2007b) found that in 5-14 year old children, dietary selenium was positively associated with plasma selenium levels (p=0.006) after multiple regression, however a study performed by Lane et al. (1983) in female elderly subjects (age range: 65-92 years) found that there was no correlation between plasma selenium and dietary selenium intake (r_p=0.13).

The myfood24 tool used in this study is a comprehensive 24-hour dietary recall assessment, making it a more substantiated method to collect dietary information compared to food frequency questionnaires (FFQ). The variety of food options available on the myfood24 programme allows diverse groups with varied food intakes to use the tool accurately, building recipes using specific ingredients when required. The minimal burden placed on users also means it is easy to conduct and simple to understand (Wark et al., 2018). However, whilst dietary recalls are one of the most feasible methods of collecting dietary data, they are still prone to issues. Participants may inaccurately recall food items as this method relies on memory. In addition, users may subjectively report food items and portion sizes, introducing the risk of recall bias (Shim et al., 2014). This could lead to inaccurate dietary selenium calculations.

Food importation and the bioavailability of selenium within foods also has the ability to drastically affect accurate assessments of dietary selenium status. The selenium content of food imported from different geographical areas can vary in selenium concentration due to properties of the soil effected by factors such as pH, rainfall and rock type (discussed in sections 1.1.2 and 1.1.3). This means that country specific

nutritional food tables tend to be inaccurate (Stoffaneller and Morse, 2015). As a result, it may be that the default selenium intake values calculated within the myfood24 tool may not be representative of the true selenium intake. Food composition also has an influence on the selenium bioavailability. For example, whilst fish produce is known to be a good source of selenium, it also contains high levels of heavy metals such as mercury and arsenic. Interactions between these elements result in strong inorganic complexes between selenium and these elements, making selenium unable to solubilise in the body. This results in a decrease in bio-accessibility needed for further metabolic processes after absorption (Thiry et al., 2012; Navarro-Alarcon and Cabrera-Vique, 2008). Whilst myfood24 may report a selenium value from known food tables, the concentrations in the blood may not reflect this as the selenium is unable to be utilised. Selenium from potential supplementation use was also not calculated in the dietary estimates. With the majority of women taking supplements in this study, dietary intake may have been underestimated resulting in inaccurate calculations of selenium intake. A combination of these drawbacks may well have been a factor in the lack of correlation seen between dietary selenium assessment and biomarker status as seen in this study

Dietary data taken in this study is a short term assessment of dietary intake over three, 24 hour periods during pregnancy. Whilst the three visits aim to give a good representation of normal dietary consumption, it would have been beneficial to gather dietary data for a longer duration of time to assess longer-term selenium intake. For example, the day-to-day variation in dietary intake of certain selenium containing foods i.e. nuts and fish may not be captured in a 24-hour recall if eaten a few days earlier (Thomson et al., 2007b). Instead of using serum selenium which is a short term indicator, whole-blood, hair and toenail samples are thought to give a better longer-term evaluation of selenium concentration (Rayman et al., 2015). Assessing one of these matrices with longer term dietary data may have been more beneficial approach to examining an association between dietary selenium and body selenium status as changes in selenium status are more apparent long-term. As well as measuring serum selenium, it would be useful in future work to identify whether GPx3 and SEPP1 correlate with dietary selenium. These key biomarkers are thought to give a better indication of selenium status, reflecting the amount of selenium required for normal selenoprotein synthesis (Thomson, 2004). It would have also been advantageous to know the specific food groups consumed by this cohort of women using the myfood24 tool i.e. fish, meats, vegetables. Food sources have varying selenium bioavailability due to a wide range of factors i.e. the speciation of selenium contained in the food (organic or inorganic selenium – section 1.1.4). Organic

selenium tends to be more bioavailable, has a greater retention and accumulates more readily in organs and tissues compared in inorganic selenium sources (Thiry et al., 2012). By deducing which foods were eaten, it may indicate which type of selenium speciation was probably absorbed, thus reflecting in the serum selenium levels. For example, meat has higher levels of bioavailable SeMet and Sec meaning we would anticipate higher serum selenium/biomarker concentrations.

4.5 Selenium and Birthweight

Whilst the assessment of selenium and pregnancy related complications such as preeclampsia and pre-term birth have been explored in the UK (Rayman et al., 2003; Rayman et al., 2014; Rayman et al., 2011), the effect of selenium concentration on birthweight had yet to be studied. Investigating for the first time in a UK based population, it was found that in early gestation (visit one), serum selenium levels were a significant predictor of birthweight (adjusted regression β=15 g, 95% CI 2 to 30 g, p=0.02). This was in contrast to the latter two visits where no such associations were found. The associations between selenium and birth weight in the prior literature give conflicting results. Bogden et al. (2006) found that USA mothers with selenium concentrations in the lowest decile during the early stages of pregnancy, predicted low birth weight in full term infants. Similar results were also found in a Japanese study. Tsuzuki et al. (2013) found that maternal selenium concentration at the third trimester was positively associated with birthweight (p=0.015). However, a study in Polish women also assessed selenium status of the mother and the resulting birthweight of the neonate at point of delivery (third trimester). It was found that maternal selenium levels at this point had no association with low birth weight (Wasowicz et al., 1993). A case controlled study in Iran found similar results; selenium and low birth weight were not significantly associated (Mohammadzadeh et al., 2009).

It is well established that thyroid hormones are important in foetal development as seen by the presence of thyroid hormone receptors and selenium containing DIO in early gestation within the foetal brain (Chan et al., 2002). A study conducted by Chan et al. (2003) found that mRNA expression of DIO2 and DIO3 was significantly elevated throughout the first trimester compared to the latter two trimesters, signifying the importance of DIO during early gestation. Whilst it has been deduced that DIO expression is probably sufficient in this cohort of women for optimal thyroid hormone supply in part due to the selenoprotein hierarchical system, it is still deficient for optimal expression of other important selenoproteins such as GPx3 and SEPP1. The

association between GPx3 and birthweight gave similar conclusions as seen for serum selenium (see Table 25). During the first visit, GPx3 was significantly associated with birthweight (unadjusted, *p*=0.019), whereas results from the second and third visits found no associations. As described earlier, sufficient selenium levels are required for optimal GPx3 expression. In this study, it was found that selenium was inadequate for GPx3 expression, resulting in lower GPx3 concentration giving the potential for weakened antioxidative defence mechanisms (Mistry et al., 2008). The reduction in GPx3 concentrations early in pregnancy could affect foetal growth due to the overexposure of ROS, causing extensive DNA and lipid damage during the most sensitive trimester (Mistry et al., 2012). After this point, the increased transportation of SEPP1 across the placenta (specifically in the last trimester) could increase the stores of selenium which the foetus is known to accumulate in its vital organs (Zachara et al., 2001). This increase may indicate why there was no such associations during visits two and three.

The reasons why we have seen a significant association between selenium and its biomarkers at the first visit only is not inherently clear. With conflicting results from previous studies (as mentioned), further research is required to fully determine the effects of low selenium during pregnancy and its influence on birth weight. This will enable us to discover, understand fully and confirm the mechanisms of selenoprotein expression and function during early gestation and whether it has a significant impact on foetal clinical outcome.

4.6 Strengths and Limitations

This study has its strengths and limitations. One of the biggest strengths lies within its design. Women agreed to partake in detailed data collection periods where anthropometric assessments, blood/urine samples, questionnaires and food diaries were all taken. Assessments for each woman in this small cohort was successfully collected across the three visits spanning gestation (i.e. 3x blood samples, 3x urine samples, 3x questionnaires and 3x 24-hour dietary recalls were all collected). This meant we had the ability to attain a true reflection of changes across the course of pregnancy– a feat which improves upon the methodology of other studies such as Knight et al. (2017) and Santos et al. (2017) who only had the ability to assess iodine concentrations during the third trimester and selenium concentrations at time of birth, respectively. This has allowed us to investigate a wealth of novel questions in this study. For example, no prior UK study has investigated associations between selenium and birthweight throughout the entirety of pregnancy, nor has one

examined correlations between dietary selenium and serum selenium status. All future work using this data will have the ability to investigate other important, contemporary questions.

Seventy participants were successfully retained throughout the project. This is a key strength, however it has been potentially limiting. This study has similar participant numbers to other studies, such as Mistry et al. (2008) and Bath et al. (2014) who analysed data from 74 and 100 participants, respectively. With the strong design allowing multiple time points across pregnancy and the sheer amount of high quality information collected at each visit, to have 70 participant complete the study with a full set of data reflects a very successful recruitment and retention process. However, despite having a large quantity of data, the participant number is relatively small and does increase the risk of unwanted variability and bias which has resulted in the study having less statistical power than originally hoped.

The cohort of pregnant women in this study were recruited in the UK city of Bradford. As a highly diverse area, it has the ability to give a unique assessment of how differing factors such as ethnicity, socioeconomic status and environmental factors affects micronutrient status throughout pregnancy. Whilst this cohort and its large amount of diversity may not be truly representative of the British population unlike others such as the 'Avon Longitudinal Study of Parents and Children (ALSPAC)' cohort, ours is still generalizable, novel and could be classed as a measure of the changing population status as seen throughout certain areas of the UK within the past 20-30 years.

During recruitment, participants were informed on the project aims and what the effect iodine can have during pregnancy. In turn, the heightened awareness of how these micronutrients may impact foetal growth and development may have made them more aware of the importance of micronutrient intake during pregnancy. This may have resulted in an improved diet and additional supplementation usage. This has the potential to effect the usual selenium and iodine dietary intake values (as discussed in section 4.4), leading to an increased risk of bias. Further studies into the exact supplementation content taken across all pregnancy is warranted to determine whether high usage had an effect on both iodine and selenium concentration, i.e. many supplements listed could simply have been the recommended folic acid. To have potentially eliminated this risk, a more blinded study design may have been beneficial. Baseline controls from non-pregnant women were also not collected, which may have improved the generalisability of this study. In the absence of this data, it makes it difficult to conclude whether there was a true

modification in concentrations and relationships in comparison to a non-pregnant population. There would be no justifiable basis to potentially recommend any changes in public health guidance without this information.

The ICP-MS analyses conducted for this study were of the highest accuracy and precision. The University of Leeds participated in the CDC EQUIP global programme which aims to standardise laboratory urinary iodine quantification. During participation, our laboratory centre passed all global criterion, equalling concentrations of iodine sent by the programme using our own ICP-MS instrumentation. This ensured that all iodine results were of the highest possible accuracy. In regards to serum selenium quantification by ICP-MS, the use of two external quality controls alongside a validated CDC methodology ensured high accuracy throughout. Seronorm™ Trace Elements and UK NEQAS samples were ran in each run with results comparing extremely well to expected values. This has ensured the data obtained is as reliable possible. However, there were also some laboratory limitations within this study. During ICP-MS development for serum selenium, the most abundant selenium isotope (Se^{80}) was not quantified alongside Se^{77} and Se^{78}. This was the result of interference from $Ar^{40}Ar^{40}$ dimers within the ICP-MS plasma torch, raising the potential for inaccurate measurements by mistakenly detecting dimers as Se^{80}. Whilst the exclusion of quantifying Se^{80} did not raise any issues during serum selenium analysis (all external and internal QC samples were accurate as per Certificates of Analysis), it may have been beneficial to optimise the instrumentation for longer to potentially avoid excluding this Se^{80} isotope or use a different method of quantification in order to include this isotope. However, financial and time constraints meant that this was not possible.

No validatory quality controls were used in SEPP1 and GPx3 ELISA analysis due to time constrains and funding. Whilst kits were pre-validated from the manufacturer and samples were run in duplicate with strict criteria on acceptance, no incurred sample repeat analysis or inter-run precision was performed. A basic test to monitor the level of anti-thyroglobulin and anti-TPO antibodies before thyroid hormone analysis was also not conducted in these maternal samples. Anti-thyroglobulin antibodies are widely known to influence thyroid hormone concentrations, increasing the likelihood of inaccurate results (Bath et al., 2017). With no measurements taken in this study due to time, funding and laboratory practicalities, biomarker results must be analysed with caution.

4.7 Future Work

A key piece of future work would be to use a larger sample size to increase statistical power. This project has shown some interesting, novel results especially in regards to assessing selenium throughout the course of pregnancy and the effects of the micronutrient on birth weight within the UK. It would therefore be a priority to repeat this analysis with an increased power to determine more conclusively the impact these results could have on pregnant women.

Basic comparisons between the two main ethnic groups of participants (Pakistani and White-British) and their biomarker status was briefly analysed during this study, however it would be beneficial to perform further complex analysis with higher sample numbers to determine whether there is a substantiated difference between the two groups. For example, the cultural differences and its impact on dietary intake may demonstrate some important results. The south-east Asian diet tends to be unlike that of a western diet. Using the myfood24 data, it would be interesting to see firstly how micronutrient status differs between the two groups and secondly, whether particular foods gives rise to lower or higher selenium and iodine status in these populations. In addition, it would be beneficial to compare concentrations of selenium, iodine and their respective biomarkers of those individuals that have taken iodine and selenium containing supplements throughout pregnancy compared to those that have not. This would enable us to discover whether supplementation had a positive effect in raising concentrations. Myfood24recalls would also be able to distinguish in those that did not take supplementation, which foods were of greater effect in providing the necessary micronutrients.

Future work would also benefit from the refinement of existing laboratory methods alongside the use of additional techniques to help consolidate results. For example, further method development using the ICP-MS instrumentation may rectify the argon dimer interference as seen with the selenium isotope, Se^{80}. The increased sensitivity and low detection limits of a triple quadrupole ICP-MS has the ability to quantify Se^{80} if utilised correctly. With more time going forward, serum selenium precision and accuracy could be improved. In addition, the use of different quantification methods i.e. western blotting alongside ELISA analysis would confirm the precision and accuracy of GPx3 and SEPP1 concentrations.

4.8 Conclusion

This study has raised important issues regarding the nutritional status of pregnant women within the UK. Being one of few pieces of work to assess a multitude of biomarkers over the course of gestation, it is apparent that women in this cohort are both iodine and selenium insufficient according to the relevant guidelines (World Health Organisation, 2007; Thomson et al., 1977). This is consistent with other pieces research that continue to highlight concerning micronutrient deficiencies within pregnant populations. We have also shown for the first time in the UK that selenium may impact early gestation during critical periods of growth and development. With the results showing just a 10 µg/L increase in serum selenium concentration leading to as much as a 150 g increase in birthweight during the most important trimester of pregnancy, the clinical implications of this discovery could be of very high significance. Further research is warranted to consolidate these findings within a larger sample size however, in the short term it is apparent is that public health education and recommendations regarding the importance of optimal nutrition throughout pregnancy must be communicated more efficiently to mothers. Heightened awareness of the increased risks a poor diet can have on the developing foetus could ultimately help prevent adverse health complications for both mother and foetus.

Appendix

5.1 Appendix 1: HIBA Questionnaire Examples

SECTION A PARTICIPANT INFORMATION

1. Date completing questionnaire ☐☐ / ☐☐ / ☐☐☐☐

Day Month Year

2. Who is administering the questionnaire? _____

3. Language used for administration ☐ English

☐ Punjabi

☐ Urdu

☐ Other: _____

4. Interpreter used

☐ Yes ☐ No

If yes, who is the interpreter?

☐ Family or friend

☐ Other

5. How many weeks pregnant are you? ☐☐ weeks ☐ Don't know

MEASUREMENTS

6. Who is taking the measurements? _____

7. Height □□□ . □□ cms

□ Not able to take

8. Current Weight □□□ . □□ kgs

□ Not able to take

9. Resting pulse rate □□□ bpm

□ Not able to take

SECTION B BACKGROUND

This first section is about your background.

20. What is your date of birth?

□□ / □□ / □□□□

Day Month Year

21. What country were you born in?

□ England □ Northern Ireland □ Poland

□ Pakistan □ Scotland □ Czech Republic

☐ Bangladesh ☐ Wales ☐ Slovakia

☐ India ☐ Republic of Ireland ☐ Romania

☐ Hungary

☐ Other: _____

22. What country were your parents born in?

Your mother:	Your father:	
☐	☐	England
☐	☐	Pakistan
☐	☐	Bangladesh
☐	☐	India
☐	☐	Northern Ireland
☐	☐	Scotland
☐	☐	Wales
☐	☐	Republic of Ireland
☐	☐	Slovakia
☐	☐	Czech Republic
☐	☐	Poland
☐	☐	Romania
☐	☐	Hungary
☐	☐	Other: _____
☐	☐	Don’t know

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