Nutritional Interventions for Thyroid Function in Adolescent Academy Footballers

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Nutritional Interventions for Thyroid Function in Adolescent Academy Footballers

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ABSTRACT

Thyroid function is vital for health, with direct impacts on metabolic rate and energy production. Research has established that exposure to various stress factors can inhibit thyroid function and supresses circulating thyroid hormones (THs). These stress factors include maturation, fasting, poor nutritional intake and increased exercise energy expenditure (EEE). It is hypothesised that these adaptations are due to altered peripheral conversion of thyroxine (T_4) away from the active hormone triiodothyronine (T_3) and toward the inhibitor reverse-triiodothyronine (rT_3). Consequently, adolescent athletes are a unique population in the sense that they can be presented with a plethora of these stress factors concurrently, yet the combined impact of this on thyroid function has not been confirmed. The aim of this thesis was to elucidate the impact of various stress factors on thyroid function in adolescent male footballers and identify periods in maturation and the sporting season when thyroid function could be at risk, with the final intention of developing a nutritional intervention in an attempt to mitigate any suppression of THs. It was hypothesised that thyroid function would be supressed under high training loads, under low energy availability (EA), during maturation and when carbohydrate intakes were low. As such it was hypothesised that providing a nutritional load to rectify the energy and carbohydrate deficits would help to preserve thyroid hormone concentrations during periods of high stress. In Study One and Two, Liquid Chromatography–Mass Spectrometry (LC-MS) analysis was confirmed as a valid, reliable and logistical method for assessing THs in adolescents and it was suggested that body temperature and blood pressure could potentially be surrogate markers for thyroid function. Study Three showed that those in peak height velocity, with low EA and with low carbohydrate intakes were more likely to have supressed TH concentrations, particularly after a competitive football match. Study Four suggested that a post-exercise carbohydrate drink can mitigate some of the acute suppression of T_3 (<60 mins), however for longer term benefits, a more systematic approach to increase energy intake might be required. This work extends understanding of supressed TH concentrations during periods of stress and provides valuable insight into when thyroid function might be at risk within adolescent male footballers. These findings highlight the importance of adequate energy and carbohydrate intakes in adolescent athletes, particularly when undergoing bouts of heavy training and during maturation.

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List of Abbreviations

The following abbreviations have been defined in the text in the first instance:

ACTH	Adrenocorticotropic Hormone
AD	Accelerations/Deceleration Load
AITD	Autoimmune Thyroid Disease
AME	Amenorrhea
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AT	Axillary Temperature
ATP	Adenosine triphosphate
BAT	Brown Adipose Tissue
BM	Body Mass
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BP	Blood Pressure
BT	Body Temperature
CHO	Carbohydrate
CI	Confidence Intervals
CK	Creatine Kinase
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DLW	Doubly Labelled Water
DXA	Dual-energy X-ray Absorptiometry
EA	Energy Availability
EB	Energy Balance
ECLIA	Electrochemiluminescence Immunoassay
ED	Energy Deficit
EDTA	Ethylenediaminetetraacetic acid
EE	Energy Expenditure
EEE	Exercise Energy Expenditure
EI	Energy Intake
ELISA	Enzyme-linked Immunosorbent Assay
EMM	Estimated Marginal Means
EPPP	Elite Player Performance Pathway
ESS	Euthyroid Sick Syndrome
EUM	Eumenorrhea
FAT	Female Athlete Triad
FFM	Fat Free Mass
FSH	Follicle Stimulating Hormone
FT3	Free Triiodothyronine
FT4	Free Thyroxine

GAS	General Adaptation Syndrome
GH	Growth Hormone
GLUT	Glucose Transporter
GPS	Global Positioning System
HDL	High-Density Lipoprotein
HESI	Heated Electrospray Ionization
HID	High-intensity Distance
HPT	Hypothalamic-pituitary-thyroid Axis
HSR	High Speed Running
IA	Immunometric Immunoassays
ICC	Intraclass Correlation Coefficient
IGF	Insulin-like Growth Factor
IL	Interleukin-
ISAK	International Society of Advanced Kinanthropometry
LBM	Lean Body Mass
LC-MS	Liquid Chromatography–Mass Spectrometry
LDL	Low-density Lipoprotein
LEA	Low Energy Availability
LH	Luteinising Hormone
LMM	Linear Mixed Model
LOA	Limits of Agreement
LOQ	Limit of Quantification
LPA	Light Physical Activity
MAS	Maximum Aerobic Speed
MD	Match Day
MET	Metabolic Equivalent task
MHR	Maximum Heart Rate
MIT	Monoiodotyrosine
MJ	Megajoule
MPA	Moderate Physical Activity
NIS	Sodium-Iodide Symporter
NTIS	Non-Thyroidal Illness Syndrome
NUFC	Newcastle United Football Club
PA	Physical Activity
PHV	Peak Height Velocity
PL	Player Load
PP	Pulse Pressure
RED-S	Relative Energy Deficiency in Sport
REE	Resting Energy Expenditure
RMR	Resting Metabolic Rate
RNI	Reference Nutrient Intake
RPE	Rating of Perceived Exertion
SBP	Systolic Blood Pressure

SD	Standard Deviation
SE	Standard Error
SED	Sedentary Activity
SEE	Standard Error of the Estimate
SEM	Standard Error of Mean
SGLT	Sodium-Glucose Co-transporter
SPD	Sprint Distance
SPE	Solid Phase Extraction
SRM	Selected Reaction Monitoring
TACITUS	Thyroid Allostasis in Critical Illness, Tumours, Uraemia and Starvation
TBG	Thyroxine Binding Globulin
TD	Total Distance
TE	Typical Error
TEE	Total Energy Expenditure
TES	Testosterone
TH	Thyroid Hormone
TL	Training Load
TR	Thyroid Hormone Receptor
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone/Thyrotropin
TT3	Total Triiodothyronine
TT4	Total Thyroxine
UCP	Uncoupling Protein
VHSR	Very High-Speed Running
VO_2	Oxygen Uptake
VPA	Vigorous Physical Activity
VT	Ventilatory Threshold
T4	Thyroxine
T3	Triiodothyronine
rT3	Reverse-triiodothyronine
T2	Diiodothyronine

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others. The work was done in collaboration with Newcastle United Football Club.

Any ethical clearance for the research presented in this thesis has been approved. Approval was sought and granted by the Faculty Ethics Committee.

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Signature:

Date: 11/03/2021

Chapter 1:
Introduction

1.1 Introduction

Over recent years the interest in the interaction between energy intake (EI), exercise and health has gained traction with the recognition of 'relative energy deficiency in sports' (RED-S) and the impact of low energy availability (LEA) on physiological function (Elliott-Sale et al., 2018; Mountjoy et al., 2014). Chronic periods of LEA can result in impaired growth and maturation, reduced bone mineral density, delayed sexual maturation, suppressed immune system and reduced thyroid function (Elliott-Sale et al., 2018; Loucks et al., 2011; Mountjoy et al., 2014). This is of specific importance to adolescent athletes, including footballers, as in conjunction with increased training loads (TL) and physical demands, they are undergoing periods of rapid biological growth and maturation resulting in physiological, anatomical and metabolic changes (Malina et al., 2015), with these changes substantially influencing the players nutritional requirements (Desbrow et al., 2014). The energy demands of growth and maturation are reported to be approximately 5 kcal per gram of body mass (BM) gained (Millward et al., 1976; Torun, 2005), this combined with increased BM are incorporated into nutritional recommendations for adolescent and paediatric athletes. However, nutritional intakes of adolescent footballers are often significantly below reference guidelines, inducing substantial energy deficits (EDs) and subsequent LEA (Briggs et al., 2015a; Morton, 2019; Russell & Pennock, 2011). Energy requirements are dictated by energy expenditure (EE), with total energy expenditure (TEE) comprised of basal metabolism (typically 45-75% of TEE), activity energy expenditure (the most variable component), thermic effect of food (typically 10% of TEE) and the energy cost associated with growth. Studies have quantified TEE and energy requirements within adolescent footballers using global positioning system (GPS) metrics, accelerometery and most-recently doubly labelled water (DLW) (Briggs et al., 2015a; Cherian et al., 2018; Morton, 2019; Russell & Pennock, 2011) which provides great insight into the activity energy requirements of athletes.

Despite it being the largest component of TEE, limited data exists on the basal metabolism of adolescent footballers, with only one recent study quantifying resting metabolic rate (RMR) (Hannon et al., 2020). Thyroid hormones (THs) are one of the largest determinants of basal metabolism, correlating with EE and BM (Mullur et al., 2014). To

date, however, there are no studies on the combined impact of growth, maturation and physical activity (PA) on thyroid function in adolescent footballers. There are five key THs; Thyrotropin/thyroid stimulating hormone (TSH), thyrotropin releasing hormone (TRH), thyroxine (T_4), triiodothyronine (T_3), reverse-triiodothyronine (rT_3) and the diiodothyronines (T_2) all dictating basal metabolic rate (BMR). Existing research recognises the suppression of thyroid function during times of stress, irrespective of the stressor, predominantly through a reduction in T_3 and increased production of rT_3 (Chatzitomaris et al., 2017). In adolescent footballers, stress factors include growth and maturation, physical training, inadequate nutritional intakes and psychological stress. Of specific interest to this thesis is the decline in T_3 reported around pubertal growth and maturation (Rubenstein et al., 1973) with concurrent reduction in RMR (Mostazir et al., 2016), the impact of LEA, EDs and periods of intense training (Heikura et al., 2017; Kanaka-Gantenbein, 2005; Loucks et al., 1998; Loucks & Callister, 1993; Loucks & Heath, 1994; Loucks & Thuma, 2003).

Although there are links to supressed thyroid function in LEA in the RED-S literature, the direct implications are less clear. Moreover, the threshold at which thyroid function is altered due to reduced EA has not been directly established. Rectifying this could provide valuable insight into the energy requirements of athletes, including adolescent footballers. Additionally, there are limited data on how acute alterations in EA impact on thyroid function that could impact on recovery, injury and future athletic performance (Nicoll et al., 2018). As such, it is important to identify practical methods to monitor thyroid function as a marker of health and EA. Furthermore, it is a priority to examine the concurrent demands of training and growth and maturation on thyroid function in adolescent footballers. The evaluation of nutritional intakes could provide valuable interventions to improve or preserve thyroid function at times when it might be at risk.

1.2 Thesis Purpose and Objectives

Few studies have evaluated the impact of exercise, maturation and nutritional intake on thyroid function. Therefore, the overarching aim of this thesis is to investigate the impact of maturation, TL and nutritional intake on thyroid function in elite adolescent male footballers. This aim is systematically addressed over four experimental chapters, which

set out to answer the main research question for this thesis; *"is thyroid function at risk in adolescent footballers and, if so, how can we preserve it?"* and meet the following objectives:

1. To determine the most appropriate methods of assessing thyroid function in adolescent athletes within the applied environment by establishing validity and reproducibility of potential approaches *(Chapters 4 and 5).*
2. To examine the relationship between maturation, TL, EE and EI on thyroid function in adolescent male footballers. This will specifically allow the identification of periods in which thyroid function might be at risk and accordingly outline the associated risk factors and inform subsequent interventions *(Chapter 6).*
3. To investigate the efficacy of a nutritional intervention to attenuate the acute impact of stress and preserve thyroid function in adolescent male footballers *(Chapter 7).*

Chapter 2:
Literature Review

2.1 Introduction

This chapter aims to highlight the pertinent research that has led to the aims of each individual experimental chapter in this thesis. The review begins discussing the role of the thyroid and THs in metabolic rate before critically evaluating current methods of assessing thyroid function in different populations. The review then discusses multiple demands faced by adolescent footballers referring to both RED-S and Hans Seyle's General Adaptation Syndrome (GAS). In support of this, the physiology of growth and maturation will be discussed as well as TL and nutritional intake in this population. Finally, potential nutritional approaches to improve thyroid function will be evaluated and an overview of the present thesis outlined. Where possible, the literature involves the population of interest, however due to the novelty of evaluating adolescent footballers with a focus on thyroid function, there are gaps within the literature where data from other populations has been evaluated.

2.2 Energy for Life – Metabolic Rate

Energy is required for life, without it we would cease to exist, and without adequate energy we are incapable of performing to our desired capacity, recovering sufficiently and adapting to increased demands (Picard et al., 2018). Mitochondrial function drives cell energy and thus dictates metabolic rate and in turn health and athletic performance (Mullur et al., 2014). Problems with low metabolic rate manifest in multiple ways including complications with skeletal development, cardiovascular and muscle function (Bassett & Williams, 2016; Danzi & Klein, 2014; Lombardi et al., 2015; Razvi et al., 2018), as such adequate energy is vital for systemic health (Mullur et al., 2014).

Despite the clear importance of metabolic rate in bodily function, health and, by extension, athletic performance, the maintenance of an adequate metabolic rate is largely under investigated. Although adaptations in metabolic rate under varying conditions have been reported, there is a lack of acknowledgment for the subsequent impact on health, athletic performance and recovery. Key mediators of BMR are BM, body composition, sex, age and hormonal status (Johannsen et al., 2012; Kim, 2008; Mullur et al., 2014; Wrutniak-Cabello et al., 2001). However, this thesis will focus mainly on hormonal

status, in conjunction with the other factors, by evaluating the impact of stress factors on THs. The role of the thyroid in energy management and maintaining metabolic rate is well documented with THs having direct effects on EE and profound effects on many vital bodily functions including growth, maturation and metabolic regulation (Mullur et al., 2014). Furthermore, evidence of decreased neuromuscular performance and recovery is reported with reduced T_3 (Nicoll et al., 2018; Tornberg et al., 2017). As such, suboptimal TH concentrations are not conducive to overall health or athletic performance (Brent, 2012).

2.3 The Hypothalamic-Pituitary-Thyroid Axis

The thyroid gland (referred to as 'thyroid') is a bi-lobular endocrine gland positioned in the anterior of the neck inferior to the larynx and is part of the hypothalamic-pituitary-thyroid axis (HPT axis) (Gard, 2002; Mohebati & Shaha, 2012). The HPT axis incorporates the hypothalamus, pituitary, adrenal and thyroid glands and associated hormones to maintain normal concentrations. This includes THs, and in turn the HPT axis is essential for biological function of most tissues (Chatzitomaris et al., 2017; Fekete & Lechan, 2013). The HPT axis is regulated by a classic endocrine negative feedback loop (Figure 2.1). However, the complexity of multiple interactions is apparent as well as the breadth of internal and external factors that can mediate the release of TH, ultimately impacting on metabolic rate. Moreover, any deviations from the norm at any point in the axis has a consequence. Therefore, due to the array of factors impacting on the HPT and the role it plays in keeping an organism in balance, it has been hypothesised that the role of the thyroid and the synergistic relationship with other glands goes beyond the realm of homeostasis and is in fact an extension of the paradigm termed 'allostasis' (Chatzitomaris et al., 2017; McEwen & Wingfield, 2003), discussed in 2.6.

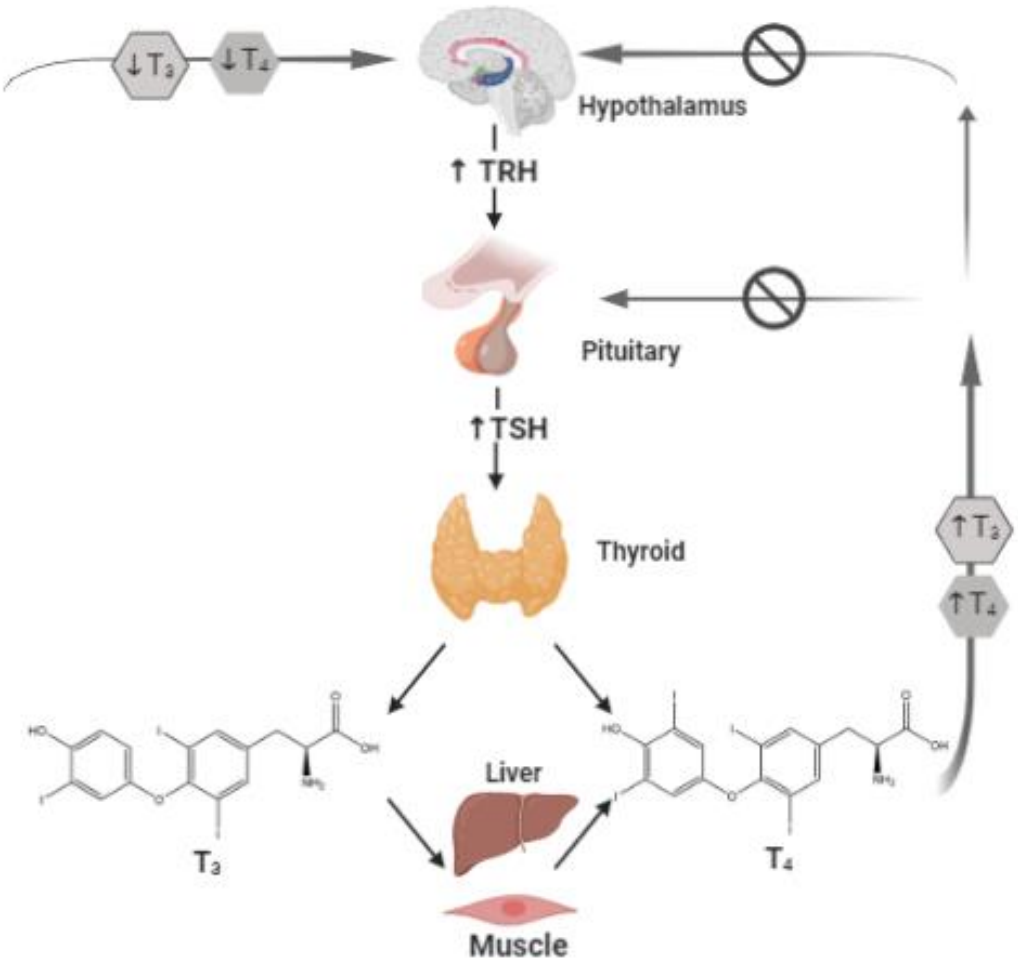

Figure 2.1 The HPT Axis. T3, triiodothyronine; T4, thyroxine; TRH, thyroid-realising hormone; TSH, thyroid stimulating hormone.

2.3.1 Thyroid Hormones

The HPT axis is responsible for the production and secretion of multiple hormones and steroids, including the tyrosine-based THs produced and secreted by the thyroid gland (Wang & Stapleton, 2010). The THs are an underlying neuro-hormonal system regarded as the key endocrine controller of metabolic rate, independent of BM and body composition, to the extent that prior to advances in TH quantification, thyroid function was inferred from RMR (Harper & Seifert, 2008; Johannsen et al., 2012). Although structurally different, all THs are iodine and tyrosine structures, accordingly, dietary iodine intake can alter TH metabolism (Aghini-Lombardi et al., 1999; Canaris et al., 2000; Tunbridge et al., 1977). Iodine is initially transported into the follicular cells by the sodium-iodide symporter (NIS) before being oxidised by peroxidase allowing for subsequent covalent boding to tyrosine within the thyroglobulin to form either

monoiodotyrosine (MIT) or T_2. A further coupling of one MIT and one T_2 molecule or two T_2 molecules results in the formation of T_3 or T_4 respectively (Bizhanova & Kopp, 2009; Dohan et al., 2003; Gard, 2002) (Figure 2.2).

It is postulated that T_3 increases metabolic rate by increasing mitochondrial respiration and subsequently increases carbohydrate metabolism and the synthesis, mobilisation and degradation of lipids whilst also stimulating protein and bone synthesis (Gard, 2002; Mullur et al., 2014). Therefore, THs play an essential role in biological activity in almost every tissue, having a pleiotropic effect on multiple organs (Brock et al., 2012; Kim, 2008; Louzada & Carvalho, 2018; Mullur et al., 2014; Silva, 2003; Van den Beld et al., 2005). The importance of these hormones is highlighted when complete absence of T_3 reduces resting energy expenditure (REE) in excess of 30%, potentially due to the impact of THs on obligatory thermogenesis (Silva, 2003). Therefore, it is clear that as a collective, THs have a large impact on general health and athletic performance. However, all THs have varying structures and different effects on the body, as well as subsequent actions on related hormones.

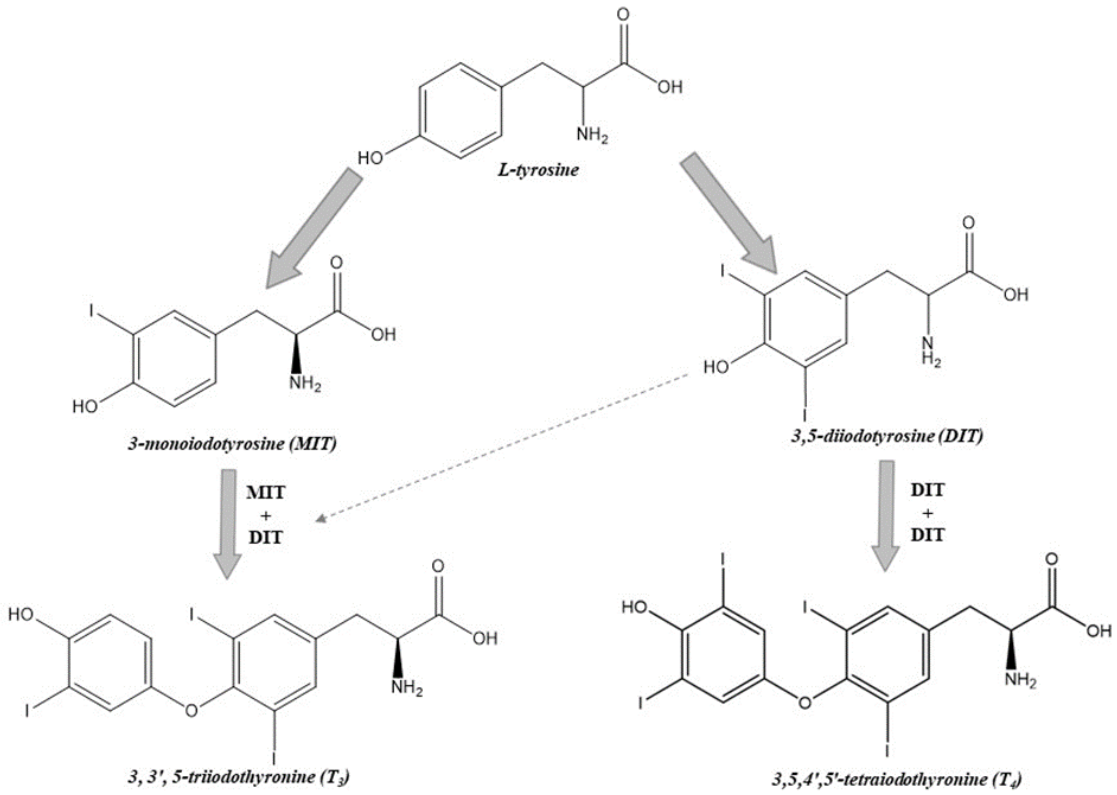

Figure 2.2 Synthesis of Thyroid Hormones.

2.3.1.1 Thyrotropin Releasing Hormone (TRH)

Thyrotropin releasing hormone is secreted by the medio-basal hypothalamus and down regulated by T_3 (Louzada & Carvalho, 2018; Mullur et al., 2014). Following secretion, the leptin-induced TRH acts upon the pituitary gland to bind to TRH receptors on the thyrotropes, increasing intracellular cAMP and subsequently regulating the synthesis, release and biological activity of TSH by the anterior pituitary gland (Abdalla & Bianco, 2014; Fekete & Lechan, 2013; Mullur et al., 2014; van der Spek et al., 2017).

2.3.1.2 Thyrotropin/ Thyroid Stimulating Hormone (TSH)

Thyrotropin/thyroid stimulating hormone is a glycoprotein secreted by the thyrotrope cells of the anterior pituitary gland with a short half-life of 5-60 minutes (Chatzitomaris et al., 2017). Despite not being secreted by the thyroid, TSH is still classed as a TH due to the direct regulation of synthesis and secretion of the other THs through the HPT axis (Li et al., 2014). Following the increase in intracellular cAMP, TSH secretion increases, binds to a G protein-coupled TSH receptor on the thyroid follicular cell, and stimulates

the production of the 'pro-hormone' T_4 (Chatzitomaris et al., 2017; Mullur et al., 2014). Accordingly, TSH regulates T_4 and T_3 production by mediating the uptake of iodide into the thyroid through increasing the activity of NA^+/K^+-ATPase and, in turn, the rate of binding of tyrosine (Brent, 2012).

Due to direct stimulation of TH production by the thyroid and TSH, it is generally believed that THs, specifically T_4 and T_3, have an inverse log-linear relationship with TSH and as such TSH is often used for diagnosis of thyroid disorders (Abdalla & Bianco, 2014; Jonklaas et al., 2014; Mullur et al., 2014). However, due to the complexity of the HPT and external influencing factors on TH production (Mullur et al., 2014), this assumption can lead to problems with diagnosis (Chatzitomaris et al., 2017; Jonklaas et al., 2014). For example, under times of LEA when T_4 production is adequate, T_3 is still supressed, often with elevated rT_3 (Beyleroglu, 2011; Hackney et al., 2012; Loucks & Heath, 1994; Mastorakos & Pavlatou, 2005).

2.3.1.3 Thyroxine (T_4)

Thyroxine (T_4) is the primary secretion of the thyroid (Mullur et al., 2014), synthesised at a much greater proportion than T_3 in the thyroglobulin molecules (Wang & Stapleton, 2010). The extent of this is uncertain, reported to be in a 3:1 ratio (Wang & Stapleton, 2010) but has been reported to be as high as 80% (Maia et al., 2011), or an 11:1 ratio (Abdalla & Bianco, 2014), with inter-individual disparities potentially accounting for these differences. Importantly, due to its lower binding affinity to thyroid hormone receptors (TR), T_4 is less biologically active than T_3 and in turn is often referred to as a 'prohormone' (Abdalla & Bianco, 2014; Brent, 2012). Subsequently, T_4 must be peripherally converted to the highly active T_3 or to a lesser extent T_2, to substantially increase metabolic rate (Chatzitomaris et al., 2017). Despite having less of a direct effect on mitochondrial oxygen consumption and metabolic rate (Wrutniak-Cabello et al., 2001), and regarded as the inactive TH, T_4 is heavily involved in the regulation of the HPT axis and is required for the production of T_3. Notably, under extremely high concentrations, T_4 does have the affinity to bind to TH receptors (TR) and elicit biological effects (Brent, 2012), however this is beyond most physiological levels and therefore has limited implications in vivo (Abdalla & Bianco, 2014).

2.3.1.4 Triiodothyronine (T3)

In contrast to T_4, T_3 has approximately a 10-fold greater binding affinity to the TR (Abdalla & Bianco, 2014) and therefore a greater impact on mitochondrial oxygen consumption (Wrutniak-Cabello et al., 2001). Triiodothyronine has a pleiotropic impact on tissues, increases metabolic rate and inhibiting further release of TSH. Circulating T_3 is derived from multiple sources with ~20% (~5 ug/day) secreted directly from the thyroid (Abdalla & Bianco, 2014; Chatzitomaris et al., 2017), with the majority produced by deiodination, sulfation, glucuronidation and ether-link cleavage (van der Spek et al., 2017) in peripheral tissues (mostly the liver and brown adipose tissue) (Abdalla & Bianco, 2014; Chatzitomaris et al., 2017; Mullur et al., 2014; Wang & Stapleton, 2010). It is estimated that healthy adults produce ~30 ug of T_3/day (Abdalla & Bianco, 2014) with most of this T_3 bound to carrier proteins, specifically T_4 binding globulin (TBG), transthyretin and albumin, with <0.4% being unbound (FT_3) (Abdalla & Bianco, 2014).

In healthy individuals, T_3 remains relatively stable despite its short half-life of 12-18 hours (Abdalla & Bianco, 2014; Chatzitomaris et al., 2017; Mullur et al., 2014). However, this is reliant on individuals having healthy thyroid and liver functions. If any of these factors are not functioning optimally, for example under stress conditions, T_3 can be unstable and have significant fluctuations both chronically and acutely throughout a life-time (Rubenstein et al., 1973; Taylor et al., 2017). This has been shown during surgical procedures (Michalaki et al., 2001), following bouts of physical activity (Beyleroglu, 2011; Hackney & Dobridge, 2009) and during LEA (Loucks & Callister, 1993).

2.3.1.5 Reverse-triiodothyronine (rT_3)

Peripheral conversion of T_4 does not only result in the formation of T_3 but, dependant on the position of the removed iodine molecule, the inactive metabolite rT_3 can be formed. Removal of an iodine molecule from the inner (5')-ring through deiodination by the type 3 iodothyronine deiodinase enzyme (D3) forms rT_3 (Maia et al., 2011). The structure of rT_3 results in a high binding affinity to TR and therefore rT_3 is a competitive inhibitor of T_3, restricting the uptake of T_3 into the cell, supressing intracellular T_3 concentrations and thus reducing metabolic rate (Abdalla & Bianco, 2014; Brent, 2012; Holtorf, 2014; van der Spek et al., 2017). Though detrimental to cellular energy and therefore systemic health, the production of rT_3 has been documented to be beneficial in times of severe LEA or starvation when the reduction in metabolic rate supports the preservation of energy for

vital bodily functions (Lopresti et al., 1991). Therefore preferential inner-ring deiodination can result in suppressed metabolic rate despite adequate TSH levels, highlighting limitations of current diagnostic criteria and the benefit of quantifying rT_3 as an indicator of altered TH peripheral conversion (Holtorf, 2014).

2.3.1.6 Diiodothyronines (T_2)

Despite T_3 being the biologically active TH and rT_3 the competitive inhibitor (Mullur et al., 2014), further cleavage of iodine atoms from both of these hormones produces several diiodothyronines: 3,5-diiodothyronine (3-5,T_2), 3,3-diiodothyronines (3-3,T_2) and 3',5'-diiodothyronine (3',5'-T_2) (Wang & Stapleton, 2010). The effect of T_2 hormones on metabolic rate is less well documented within literature than the other THs, however 3,5-T_2 is proposed to have the greatest activity of the diiodothyronines through the ability to supresses TSH levels, increase oxygen consumption, metabolic rate and lipid oxidation (Lombardi et al., 2015; Louzada & Carvalho, 2018; Wang & Stapleton, 2010).

2.3.2 Thyroid Hormone Action

Thyroid hormones can exert a multitude of physiological actions depending on the target organ (Table 2.1), with the biological impacts mediated via transcriptional regulation by steroid TRs (Cheng et al., 2010). As such, the biological effect of THs are dependent on TH concentration and the levels and type of TR present within a tissue (Anyetei-Anum et al., 2018). The TRs can be separated into two subtypes: TRα and TRβ, with alternative splicing producing six T_3-binding receptor isoforms both active (TRα_1, TRβ_1, TRβ_2 and TRβ_3) and inactive (TRα_2, TRα_3). The inactive isoforms contain a unique carboxy terminus preventing further binding of TH and inhibiting the action of the active TR isoforms (Anyetei-Anum et al., 2018; Bassett & Williams, 2016; Boelaert & Franklyn, 2005; Cheng et al., 2010).

Both TRα and TRβ are present in most tissues with expressions levels varying, indicating different functions in differing tissues. TRα have high expression rates within the HPT axis, kidney, muscle and heart, whilst TRβ are most abundant within the liver and pituitary. TRβ_2 has a unique amino acid terminus and is selectively expressed in the hypothalamus and pituitary, playing a role in TH regulation and believed to upregulating TH secretion (Bassett & Williams, 2016; Cheng et al., 2010). Similarly TRα_2 has the

greatest expression in the hypothalamus and pituitary however its antagonistic role is postulated to downregulate TH secretion (Anyetei-Anum et al., 2018). The presentation of different TR isoforms and cell specific availability of T_3 showcases the complex co-ordinated system for fine control of T_3 availability and action.

Table 2.1 Impact of Thyroid Hormones on Specific Target Systems

Target System	**Impact of Thyroid Hormones**
Metabolism	↑*BMR, heat production, protein synthesis, protein degradation, lipogenesis and lipolysis*
HPT Axis	↓ *TSH and TRH secretion*
Cardiovascular System	↓ *peripheral resistance*
Nervous System	↑ *catecholamine's sensitivity*
Skeletal System	*Regulates bone turnover*
Kidney	↑*Renal blood flow*
Reproductive System	↑*conversion of androgens to oestrogenic products* *Regulates LH/FSH pulses*

BMR, basal metabolic rate; TSH, thyroid stimulating hormone; TRH, thyrotropin releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone

2.3.3 Thyroid Hormone Metabolism

As previously mentioned, THs are mainly metabolised through deiodination and sulfation. This peripheral conversion account for ~80% of all T_3 produced, thus it is evident these pathways are essential control points for cellular thyroid action (Holtorf, 2014; van der Spek et al., 2017).

2.3.3.1 Deiodination

Deiodination, the removal of an iodine residue from the inner or outer ring catalysed and mediated by the iodothyronine deiodinase enzymes (Figure 2.3), is the major pathway of peripheral T_3 production (Abdalla & Bianco, 2014; Holtorf, 2014; van der Spek et al., 2017; Wu et al., 2005). Three deiodinase enzymes exist, D1, D2 and D3, expressed in varying concentrations within different tissues and cells including the thyroid, the liver and brown adipose tissue (BAT) either up or down regulating T_3 production and in turn

metabolism (Abdalla & Bianco, 2014; Mullur et al., 2014). Deiodinase enzymes control the bioactivity of TH by inner and outer-ring deiodination, with outer-ring deiodination activating TH and inner-ring inactivating TH (Visser, 1994).

The D1 and D2 pathways are catalysts for both thyroidal and peripheral conversion of T_4 to T_3 via outer (5')-ring deiodination of T_4 increasing intracellular T_3 concentrations (Abdalla & Bianco, 2014; Maia et al., 2011; van der Spek et al., 2017). However, due to different locations and expressions, the two pathways differ slightly. Deiodinase enzyme 1 (D1) is predominantly present within the liver, kidneys, thyroid and pituitary (Abdalla & Bianco, 2014; van der Spek et al., 2017) and can remove an iodine molecule from both inner and outer-ring of T_4. However, in comparison to the D2 pathway, it contributes a smaller proportion of T_3 (~5 ug/day and ~20 ug/day, respectively) (Brent, 2012; Mullur et al., 2014) as the preferred substrate of D1 is not T_4 but instead rT_3, sulphated T_3 and sulphated T_4. Therefore, the primary role of D1 is to degrade inactive TH and conjugated T_3 (Mullur et al., 2014; van der Spek et al., 2017; Visser, 1994). In turn D1 plays a key role in the clearance of conjugated T_3 (Mullur et al., 2014).

Deiodinase enzyme 2 (D2) is also expressed within the pituitary and is present in large amounts within BAT, the brain and liver (Holtorf, 2014; Landsberg, 2012; Silva, 2003; van der Spek et al., 2017). Similar to D1, the preferred substrate of D2 is T_4. However, D2 can only deiodinase the outer-ring and therefore is thought to mediate the majority of T_3 production in healthy adults (van der Spek et al., 2017; Visser, 1994). As such, D2 can increase intracellular T_3 production to such an extent that near 100% of TR are saturated compared to the usual 70%. This has been demonstrated during cold exposure, potentially stimulated by increases in noradrenaline that is known to stimulate D2 (Landsberg, 2012). However despite increased TR binding, serum FT_3 levels remained unaffected in this scenario (Silva, 2003). This is thought to be due to the high presence of D2 within the endoplasmic reticulum and the short half- life of TR (van der Spek et al., 2017). In turn, T_3 produced through this pathway remains within the cell for ~8 hours and only triggers biological effects in the cell it was produced (Abdalla & Bianco, 2014). In addition, the large amount of D2 within BAT results in D2 manufactured T_3 being a large mediator of thermogenesis in BAT (van der Spek et al., 2017). Due to the high presence of D2 within

the pituitary, the T_3 produced here impacts on the pituitary but not the rest of the body, however due to the endocrine responses associated with THs, this will have concurrent impacts on TSH concentrations. With this regard it is important to note that unlike D1, under stress conditions, D2 is upregulated, increasing intra-pituitary T_4 to T_3 conversion whilst the rest of the body suffers from supressed T_3 levels (Holtorf, 2014). This can have further consequences as the high intra-pituitary T_3 concentrations will inhibit systemic T_4 conversion and therefore whole body T_3 levels will remain supressed.

Unlike the D1 and D2 enzymes, D3 is involved in the catabolism of both T_4 and T_3, by catalysing the inner (5')-ring deiodination of T_4 (Maia et al., 2011; van der Spek et al., 2017) terminating TH action and depleting intracellular T_3 concentrations (Abdalla & Bianco, 2014). This is largely attained by the conversion of T_4 to rT_3, which is important in regulating circulating T_3 levels and in HPT axis regulation (Brent, 2012) and in turn D3 is positively regulated by T_3 (Abdalla & Bianco, 2014). Although D3 is present in relatively low concentrations, it is present in all tissues apart from the pituitary and is most abundant in the placenta (Holtorf, 2014; van der Spek et al., 2017). Similar to D2, D3 is upregulated during stress, increasing circulating rT_3 and supressing T_3, thus reducing the binding of T_3 to TR, reducing cellular metabolism and decreasing ischemic damage (van der Spek et al., 2017).

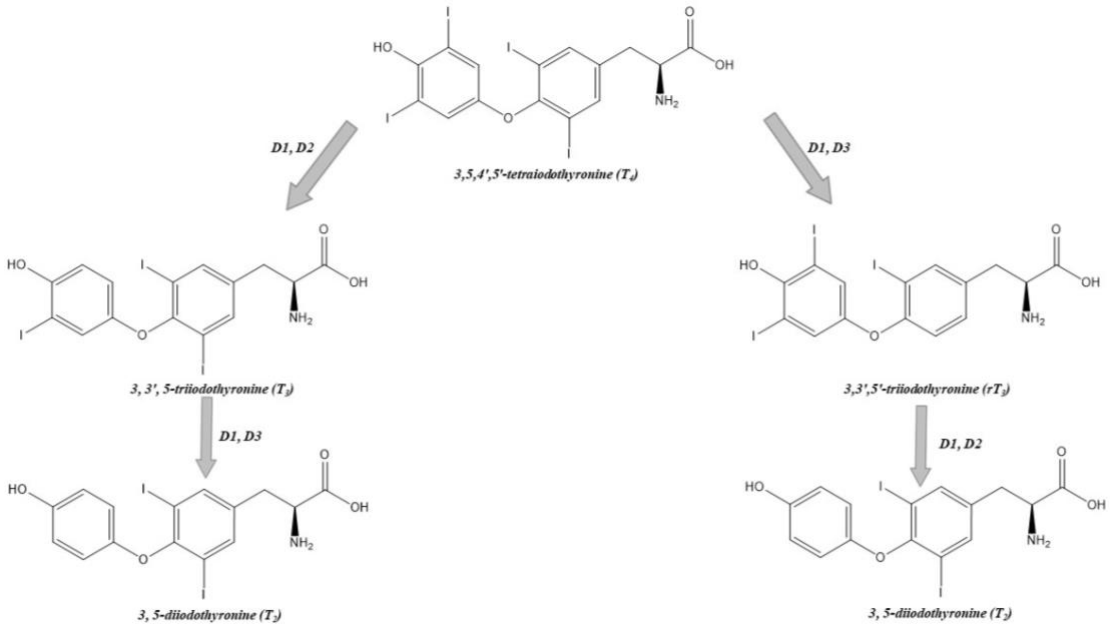

Figure 2.3 Metabolism of Thyroid Hormones by Deiodination.

2.3.3.2 Sulfation

Although the deiodination pathway is the largest contributor to peripheral thyroid hormone metabolism, the conjugation of the phenolic hydroxyl group of T_4 with sulphate or glucuronic acid results in the formation of sulphated T_4 (T_4S) and T_4-glucuronide (T_4G) respectively (van der Spek et al., 2017). Although the sulfation of the phenolic hydroxyl group of T_4 does not independently convert TH, the sulfotransferase enzymes facilitate deiodination by D3 (Wu et al., 2005), as T_4S inhibits the outer ring deiodination by D1, allowing only inner ring deiodination and subsequently the production of sulphated rT_3 (rT_3S) (van der Spek et al., 2017; Visser, 1994). Sulphated TH are biologically inactive due to the low binding affinity and are very low in human serum, suggesting the preferential excretion and degradation by D1 (van der Spek et al., 2017). Therefore, importantly even in low concentrations, stored T_3S can be utilised when D1 activity is low or T_3 levels are supressed, for example during stress-induced hypothyroidism, non-thyroidal illness (NTIS) and during foetal development (Visser, 1994).

2.3.4 The Liver and Thyroid Function

As previously discussed, multiple thyroid disorders now exist beyond that of clinically diagnosed hypo- and hyper-thyroidism, including ESS, NTIS and low T_3 syndrome. The

common presentation of these disorders include low circulating T_3 with normal or slightly elevated T_4, with this pattern generally agreed to be as a result of impaired peripheral conversion of THs (Chatzitomaris et al., 2017; Fliers et al., 2015; Gesing et al., 2012; Michalaki et al., 2001). These diseases often occur during times of heightened stress and the associated increase in catecholamine's, as well as during energy deprivation and when detoxification is a priority, for example for drug and steroid metabolism (Chatzitomaris et al., 2017; Fommei & Iervasi, 2002; Michalaki et al., 2001). As the liver is predominantly responsible for the peripheral conversion of T_4 to T_3 it is therefore postulated that issues with liver efficiency and activity of hepatic antioxidant enzyme systems is a potential cause of these disorders (Kelly, 2000; Malik & Hodgson, 2002). The liver is one of the most metabolically active organs not only critical in maintaining whole body lipid, glucose and energy metabolism but also a major organ for detoxification of exogenous and endogenous substances (Chiang, 2014; Kelly, 2000; Sendensky & Dufour, 2011). Therefore, a low energy liver can inhibit T_4 conversion due to the prioritisation of other pathways, including: blood glucose maintenance, lipid metabolism and detoxification (Chiang, 2014; Mullur et al., 2014; Sendensky & Dufour, 2011). Stress is characterised by increased circulating adrenalin and cortisol, however, increased circulating cortisol inhibits T_4 deionisation further (Hackney et al., 2012; Límanová et al., 2009). This results in a detrimental cycle of poor metabolism and liver function (Kanaka-Gantenbein, 2005; Mullur et al., 2014; Tolfrey et al., 2017), which becomes increasingly likely during times of high stress (Mancini et al., 2016).

Within sporting populations there is an additional factor impacting on T_3 levels and therefore metabolic health due to increased TL, amplified with the addition of high-intensity intermittent exercise (Hackney et al., 2012). Furthermore, significant energy deficits (EDs) have been reported within adolescent male academy footballers (Briggs et al., 2015a) which can further inhibit T_3 production (Kanaka-Gantenbein, 2005). It is therefore postulated that the increase in these stress factors, testosterone and the subsequent increase in hepatic antioxidant enzyme substrates, including cytochrome P450, might influence the peripheral metabolism of TH (Kelly, 2000; Malik & Hodgson, 2002). Therefore, due to the role of the liver in TH metabolism and detoxification it could be possible that T_3 concentrations could be compromised if the liver is subject to increased chemicals, toxins or is diseased (Malik & Hodgson, 2002). This has been evidenced by incidents of suppressed T_3 with increased rT_3 in cirrhosis (Bianchi et al.,

1991; L'age et al., 1980), ESS and subclinical hypothyroidism documented in children with chronic liver disease (Ön et al., 2019) as well as a negative correlation between T_3:T_4 ratio and liver failure severity (Kano et al., 1987). Due to the concurrent factors discussed it could be hypothesised that similar effects could exist within adolescent athletes.

2.4 Diseases of Thyroid Dysfunction

Thyroid disorders are common worldwide, with greater prevalence in females (NICE, 2019) and can be subdivided into key areas including thyroiditis (the presence of thyroid inflammation), alterations in thyroid function (such as hyperthyroidism and hypothyroidism), goitre and iodine deficiency (enlargement of the thyroid gland) and autoimmune thyroid disease (AITD) thyroid autoimmunity (such as autoimmune thyroiditis and Graves' disease). Symptoms of thyroid dysfunction present in multiple ways with impacts on the skin, metabolism, and renal function as well as on the cardiovascular, nervous, respiratory and reproductive systems. In iodine-replete populations the most common cause of thyroid dysfunction is thyroid autoimmunity (Vanderpump, 2011). However, the focus of this thesis is on alternations in thyroid function, specifically T_3 suppression under varying concentrations and therefore considers hypothyroidism, both subclinical and overt, in the greatest detail.

Prevalence data of thyroid disorders within the UK is lacking due to limitations and differences with diagnostic criteria between regions, with no standardised diagnostic criteria in primary or secondary care (NICE, 2019). In 1977 2.7% and 0.23% of males and females respectively reported TSH abnormalities within the north-east of England (Tunbridge et al., 1977), substantially smaller than the 2.9% and 17.9% reported by over 60 year olds in Birmingham (Fade et al., 1991). The notable increase in prevalence might be reflected by the age of participants, as TSH was markedly higher in females over 45 year olds in the earlier study (Tunbridge et al., 1977). More recently, prevalence of all thyroid disorders has reported to impact on 5.9-11.7% of the population in the US (Canaris et al., 2000; Hollowell et al., 2002) and 9% of females in Norway (Bjoro et al., 2000). In Greece the prevalence is greater at 13.8% in a cross-sectional study of 4754 individuals, 20.4% in females (Magriplis et al., 2019), of interest to this thesis is the increased prevalence also occurring alongside increased prevalence of stress-related

disorders (including chronic perceived stress and depression) indicating the need to assess risk factors such as dietary intake and levels of stress. Alternatively, this rise could be due to previously iodine-deplete areas causing cases of goitre followed by 'silent iodine prophylaxis' throughout 1980-1990 causing a shift towards AITD (Fountoulakis et al., 2007; Zois et al., 2003)

Based on studies over the past 40 years, worldwide prevalence for clinically diagnosed hypothyroidism and hyperthyroidism was 7% and 2% respectively (Vanderpump, 2011). However, the true number of those impacted by supressed thyroid function is unknown, in part due to differences within methodologies and fundamental problems with diagnosis criteria, with a heavy reliance on TSH concentrations. In turn, a large proportion of the population could suffer from supressed thyroid function, whilst being asymptomatic and unaware they have inadequate thyroid function (Canaris et al., 2000), thus supporting the notion of screening for early detection especially in those under stress conditions, and for screening a wider range of THs.

2.4.1 Clinical Thyroid Dysfunction

Two forms of thyroid dysfunction are diagnosed clinically: hyperthyroidism and hypothyroidism. Hypothyroidism is more prevalent across whole populations compared to hyperthyroidism, at 4.6% compared to 1.3% respectively (Hollowell et al., 2002). However, due to diagnostic methods, true cases could be higher than reported. Hyperthyroidism, an overactive thyroid gland and therefore overproduction of THs, is clinically diagnosed with low TSH concentrations and high T_4 levels. In contrast hypothyroidism is the suppression of T_4 with raised TSH concentrations, with clinical manifestations outlined in Table 2.2, with those believed to have the largest impact on adolescent athletes highlighted in italics (Danzi & Klein, 2003; Danzi & Klein, 2014; Razvi et al., 2018; Surks, 2012; Vanderpump, 2011).

Table 2.2 Clinical Manifestations of Hypothyroidism and Hyperthyroidism, adapted from Muller (2016)

	Hypothyroidism	**Hyperthyroidism**
Cardiovascular System	*Bradycardia* *Blood pressure alternations* *Vasoconstriction → cold intolerance*	*Tachycardia* *Blood pressure alterations*
Respiratory System	*Dyspena*	*Dyspnea*
Metabolism	↓ *Appetite* ↑ *BM* ↓ *Plasma free fatty acids* ↑ *LDL cholesterol*	↑ *Appetite* ↓ *BM* ↑ *Plasma free fatty acids* ↓ *LDL cholesterol*
Nervous System	Memory defects Lethargy Depression	Nervousness Insomnia Hyperkinesia
Muscle	*Weakness* *Fatigue* ↑ *CK levels*	*Weakness* *Fatigue* *Muscle wastage*
Skin and Hair	Dry skin *Slow wound healing* Dry and brittle hair → hair loss	*Excessive sweating* Soft and friable nails and hair
Calcium Metabolism	*Bone frailty* Osteoporosis	Hypercalcemia
GI System	Constipation	Diarrhoea

LDL; Low Density Lipoprotein, CK; Creatine Kinase, BM, Body Mass; Italics; those with negative consequences on adolescent athlete performance

2.4.2 Alterations in Thyroid Hormones

There are a host of other less commonly known illnesses associated with altered thyroid homeostasis, including the subclinical conditions of specific interest to this thesis that result in supressed T_3. These include subclinical hypothyroidism, NTIS or euthyroid sick syndrome (ESS) and thyroid allostasis in critical illness, tumours, uraemia and starvations (TACITUS). These disorders present analogously and are often difficult to distinguish without accounting for concurrent illnesses (Table 2.3). However, these illness and subsequent abnormalities are usually reversible (Michalaki et al., 2001) and characteristically present with low levels of T_4, T_3 and impaired binding of TH to TR with

a concurrent increase in rT_3 and T_2 levels (Boelen et al., 2008; Chatzitomaris et al., 2017; Fliers et al., 2015; Gesing et al., 2012; Razvi et al., 2018; Van den Beld et al., 2005). In contrast to clinical hypothyroidism which would result in heightened TSH concentrations in response to supressed T_4, subclinical conditions might frequently go undiagnosed due to their often asymptomatic nature and the requirement for more sensitive analytical techniques, thus subclinical disorders might be more prevalent than reported (Gesing et al., 2012).

An exception to this is NTIS, which often occurs in response to an acute bout of systemic illness and is reflected in energy restriction and fasted states as a protective mechanism due to the subsequently reduced metabolic rate (Boelen et al., 2008; Chatzitomaris et al., 2017; Fliers et al., 2015; Gesing et al., 2012; Razvi et al., 2018; Van den Beld et al., 2005). Therefore, although less extreme than periods of starvation, it could be postulated that this could occur during times of LEA. In turn, it is thought that the suppression of T_3 to preserve energy could be exacerbated at times when the body is under stress, including during maturation and with intense training in an athletic population. As such, athletic adolescents could be at risk of acute or more systemic bouts of subclinical disorders, especially with the reported decline of T_3 with age (Canaris et al., 2000).

Table 2.3 Disorders Associated with Suppressed Thyroid Function

Disorder	**Clinical Manifestation**	**Alterations in TH**
Subclinical Hypothyroidism	Often asymptomatic Similar to hypothyroidism	↓ T4 ↓ T3 ↑ (minor) TSH
NTIS/ESS	Often asymptomatic Similar to hypothyroidism	↓ T4 ↓ T3 No alteration in TSH
TACITUS	Often asymptomatic Similar to hypothyroidism	↓ T4 ↓ T3 ↑ TSH (in recovery)

TH, thyroid hormones; T_4, thyroxine; T_3, triiodothyronine; TSH, thyroid stimulating hormone; NTIS, non-thyroidal illness syndrome; ESS euthyroid sick syndrome; TACITUS, thyroid allostasis in critical illness tumours uraemia and sickness.

2.4.3 Thyroid Hormones and Ageing

In healthy ageing there are adaptions in TH concentrations associated with poor health, with T_3 declining almost linearly with age, indicated in Figure 2.4 (Rubenstein & Butler, 1973). This is thought to account (in part) for the theory that ageing is the decline in structure and function over time (Kriete et al., 2010; Wallace, 2005), and in turn that diseases associated with ageing are related to the progressive decline in metabolic rate and mitochondrial dysfunction (Brand, 2000; Wallace, 2005). An important corollary to this progressive decline in T_3 with age, and a notable contribution of the work by Rubenstein et al. (1973), is the clear abrupt drop in T_3 concentrations coinciding with the mean onset of puberty with a decrease in 35 ng/dl from 10-15 y compared to an average decrease of 5.1 ng/dl for each other 10 y period. In support of this early study, a recent 10 y longitudinal study identified that EE declined around puberty when rapid growth occurred (Mostazir et al., 2016), falling by 110 kcal/day from 10-15 y in males. Notably, this study also monitored key hormones associated with EE including insulin, insulin-like growth factor 1 (IGF-1), luteinizing hormone (LH), follicle-stimulating hormone (FSH), leptin and adiponectin. Despite each hormone depicting noticeable trends over time, they were unable to entirely explain the observed decline in REE, with only insulin and leptin showing significant association but with a small effect sizes not fully accounting for the reduction in REE seen during rapid adolescent growth (Mostazir et al., 2016). Therefore, it was indicated that TH could be responsible for this significant reduction in REE at this time point.

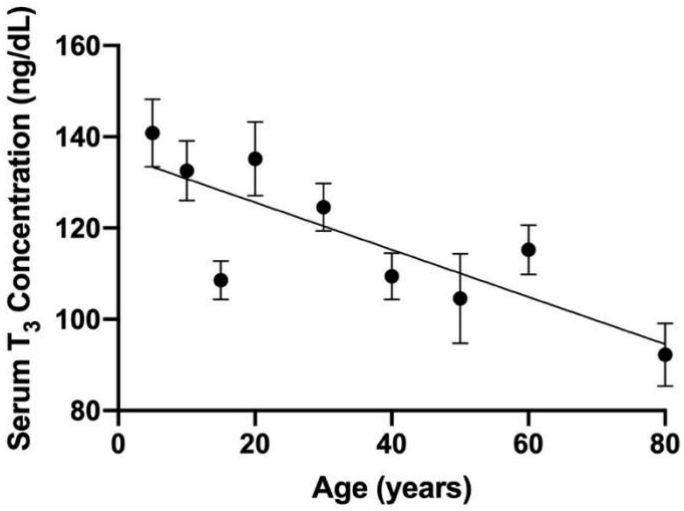

Figure 2.4. Serum triiodothyronine concentrations decreasing with age in males and females by Rubenstein et al. (1973).

A similar longitudinal study, considering EE relative to body composition and maturation assessed through Tanner stages, found that maturation was negatively related to REE independent of age, fat mass and lean mass (Sun et al., 2001). This was not significant from stage one to two, however in the latter Tanner stages (three and four), this reached significance with a 315 kJ/d and 935 kJ/d decline in REE from stage one to stage three and four respectively. However, the authors failed to assess hormonal status and as such identified no possible mechanisms; additionally, the use of Tanner stages for maturation is very subjective. Despite limitations in both studies and neither study identifying potential mechanisms, the drops in REE associated with pubertal growth do align with the decline in T_3 reported at this stage (Rubenstein et al., 1973). However, this potential mechanism is yet to be directly assessed.

A major limitation of the work by Rubenstein et al. (1973) is the use of chronological age as opposed to biological age, the former not accounting for maturation status. However, the significant drop in T_3 around the age at which most males reach peak height velocity (PHV) does align with the EE decline reported by Mostazir et al. (2016). Notably, T_4 levels did not decrease to the same extent as T_3 indicating that this decline in T_3 is related to a factor other than the thyroid. Therefore, this could be related to the peripheral deionisation of T_4 to T_3, potentially related to an iodothyronine deiodinase polymorphism, or a problem at a peripheral conversion site such as the liver. This conjecture is supported by the positive relationship between rT_3 and age, indicating a preference for rT_3 secretion ahead of T_3 production and rT_3 degradation and clearance (Van den Beld et al., 2005). In support of this, a similar significant reduction in FT_3 was reported in 884 children from age 7 to age 15 y whilst TSH increased over the same time (Taylor et al., 2017). Furthermore, individuals at a more advanced Tanner stage at age 13 y were seen to have declining FT_3 concentrations, whereas those of less advanced pubertal status still have increasing FT_3 levels.

The evidence of reduced REE and TH contradicts the more commonly regarded energy demands of growth estimated to be approximately 5 kcal/g of BM (Millward et al., 1976). Therefore, it is postulated that a 1-2% positive energy balance (EB) is required for these increased growth demands (Livingstone et al., 1992; Livingstone & Robson, 2007). These energy demands are believed to be due to additional substrates needed to develop new tissue and for the increased metabolic cost of growth (Millward et al., 1976). However,

the studies that identify the potential need for a positive EB are compounded by use of weighed dietary records to assess EB in children and adolescents. These methods have a large number of limitations, including a trend towards under-reporting of EI and an age-related bias in reporting dietary intake documented by Livingstone and colleagues (1992). Despite the proposition that a positive EB is necessary during maturation (Livingstone et al., 1992; Livingstone & Robson, 2007) this is contradictory to evidence indicating reduced circulating T_3 concentrations (Rubenstein & Butler, 1973) and the reduced REE (Mostazir et al., 2016) documented across this time. This disparity has not been previously discussed and no studies have assessed EI, expenditure and TH throughout maturation. It could be hypothesised that a fall in T_3 and REE are a consequence of the energy demands of growth not being entirely met, and the body reducing EE to preserve vital bodily functions, similar to NTIS. As such, it could be postulated that impaired hepatic production of T_3 could be due to inadequate nutritional intake and not aging itself (Van den Beld et al., 2005). Therefore, an increase in EI to meet the demands associated with puberty could alleviate this decrease in T_3, REE and associated illnesses. In turn adolescent athletes could be at increased risk of supressed T_3 due to inadequate EI (Briggs et al., 2015a) coinciding with maturation and the concurrent effect on THs. Current methodologies, however, do not easily allow for monitoring on thyroid function within this potentially at risk population.

2.5 Methods of Assessment of Thyroid Function

2.5.1 Serum Thyroid Hormone Concentrations

The most widely used diagnostic assessment of thyroid function is through the quantification of THs. Contemporary diagnosis relies on assessment of TSH (Chatzitomaris et al., 2017) with subsequent assessment of FT_4 if TSH concentrations fall outside the normal range (50-160 ug/dL). It is only in rare circumstances that further tests are carried out to assess for circulating levels of FT_3 (normal range 4-8.3 ng/dL) (Chatzitomaris et al., 2017). This has clear limitations, as individuals could have supressed T_3 remaining undetected due to TSH or T_4 within reference ranges. Despite this method of assessment being inexpensive, it can be seen to over-simplify the pathologies associated with thyroid dysfunction and in turn carries large risks of false positive and false negative results (Chatzitomaris et al., 2017).

Clinical hypothyroidism is defined as elevated TSH levels accompanied with supressed T_4, and hyperthyroidism as supressed TSH levels with heightened T_4 (NHS, 2018). However, despite the tight coupling of all elements of the HPT axis feedback loop and the expected inverse log-linear relationship between T_4 and TSH (Chatzitomaris et al., 2017; Jonklaas et al., 2014), it is possible for patients to have thyroid problems without these pathologies. For example, in conditions such as NTIS and TACITUS, low T_4 and T_3 are present with TSH within references ranges and often with elevated rT_3 concentrations (Andersen et al., 2002; Jonklaas et al., 2014; Masika et al., 2016). It has also been noted that due to the presence of D2 enzyme within the pituitary, individuals could have adequate intra-pituitary T_3 and thus reported adequate TSH levels while the rest of the body has supressed T_3 levels suggesting TSH alone cannot be used as a diagnostic tool (Holtorf, 2014). Therefore, individuals with supressed metabolic rate due to a reduction in T_3 could remain undiagnosed due to current clinical diagnostic methods. Thus, the measurement of the full suite of THs, including the ratio of T_3 to rT_3, should be utilised in diagnosis and might explain other metabolic abnormalities whilst considering the factors impacting the TSH-FT_3 relationship (Wang & Stapleton, 2010). However, a thorough study of 2673 patients evaluated the relationship between total T_3 (TT_3), total T_4 (TT_4) and TSH in the diagnosis of thyroid dysfunction and commented that, in healthy populations, FT_4 and TSH are adequate diagnostic tools, and within hyper- and hypothyroidism individuals, TT_4 and TSH are the appropriate hormones to monitor (Li et al., 2014). Finally, common diagnosis utilises one serum sample from one time point, which is not standardised amongst clinicians and therefore does not account for circadian fluctuations in TH concentrations or adaptations in response to maturation or stress (Chatzitomaris et al., 2017).

2.5.2 Reference Ranges

In addition to the limitations of only testing two of the TH, and specifically not initially assessing circulating FT_3 levels, there are concerns surrounding the reference ranges and cut off points for these hormones for diagnosis (Chatzitomaris et al., 2017). Reference ranges vary worldwide and even between trusts within the same country, causing confusion in diagnosis and treatment of thyroid dysfunction as well as within research contexts. There have been arguments made towards adjusting reference ranges with regard to narrowing the reference range (Hollowell et al., 2002; Wartofsky & Dickey,

2005) as well as the use of individual and smaller population-based reference ranges (Andersen et al., 2002; Thienpont et al., 2013b). This is largely due to the improvement in analysis techniques and the enhanced sensitivity of assays. In addition, it was noted that previous reference ranges have been skewed by individuals with AITD included in one study (Hollowell et al., 2002), thus increasing the reference range for TSH. This has resulted in the upper reference limit for TSH subsequently being reduced over the past two decades, however this is still postulated to remain too high and result in underdiagnoses of thyroid disorders (Biondi, 2013). Further to this, reference ranges are set in 'healthy normal' conditions of non-pregnant, fed, rested individuals and therefore do not account for differences in response to allostatic adaptations (Chatzitomaris et al., 2017).

Numerous authors have outlined the importance of narrower individual reference ranges for TH testing due to the large biological variation associated with these tests (Andersen et al., 2002; Kapelari et al., 2008; Thienpont et al., 2013b; Williams et al., 1978). As early as 1978 it was reported that the individual range is significantly smaller than the group variation amongst euthyroid patients (Williams et al., 1978). More recently this has been quantified. Anderson et al. (2002) found that individuals' test results over a 12 month period, varied within 50% of the distribution for the group indicating that intra-individual variation is maintained within relatively narrow limits in comparison to the large inter-individual variation. However, with a sample size of only 16 and a large variation in age, large group variation could be expected. In support of Anderson et al. (2002), a more comprehensive review by Theinport et al. (2013) reported that individual variation of TSH, TT_4 and TT_3 were narrow compared to laboratory reference ranges, with individual variation of TSH half the width of reference ranges. Furthermore, individuals with concentrations within the upper and lower reference ranges were most at risk of inaccurate results, clearly detrimental to diagnosis (Thienpont et al., 2013a). Accordingly, population based reference ranges do not account for the documented large inter-individual variations, therefore, conventional population-based reference ranges are not appropriate for assessing if an individual's fluctuations are abnormal for their own range (Andersen et al., 2002).

Ideally, individual reference ranges would be used in diagnosis of thyroid related conditions, however this is not feasible due to the high number of tests required to identify an individual's reference range. Theinport (2013) concluded that approximately 126 tests would need to be conducted for each individual. This is supported by Anderson et al. (2002) who reported >25 tests would need to be conducted for 95% accuracy for TSH (Andersen et al., 2002) which could provide greater insight than population reference values. The amount of tests required for an individual reference range for T_3 however remains unknown, and due to the complexity of the inverse log-linear relationship between TSH and the other THs, this could not be solved by stratification of TSH results (Andersen et al., 2002).

It could be appropriate to identify smaller population reference ranges in relation to known determining factors including age and sex which has been debated over the last four decades. Williams et al. (1978) made the assumption that individuals of the same age and sex would possess similar reference values and ranges. Their study supported the sex differences predicted, with T_4 significantly higher in women than men. However, they found no significant age effect for T_4. A major limitation of this study was the lack of assessment of other THs, specifically T_3. In addition to this, despite the large sample of 1105 healthy patients, the age groups chosen did not reflect known pubertal fluctuations previously reported by Rubenstein et al. (1973). Therefore, the adaptation of age categories could dramatically impact on the reported results. Despite these limitations and the lack of significant age effect on T_4 Williams et al. (1978) supported the use of individual reference values and encouraged the use of specifically defined reference values in relation to age and sex to replace conventional general population ranges.

A later study by Sappin et al. (1998) evaluated FT_3 within 149 euthyroid patients and found no sex effect of FT_3 levels. However, an inverse FT_3 age relationship in the euthyroid subjects was reported and thus they outlined reference ranges for age groups <20 y, 20-60 y and >60 y, as well as further supporting the earlier reported abrupt decline in T_3 during puberty (Sapin et al., 1998). Due to the lack of TSH and T_4 data it is not possible to draw direct comparisons to earlier work (Williams et al., 1978) or comment on the potential physiological mechanisms behind the TSH-T_3 relationship. More

recently, paediatric reference values were more closely assessed supporting the importance of narrower age reference ranges, particularly until adulthood is reached (Kapelari et al., 2008). From 0-18 years old, no sex difference was reported for TSH or FT_4, however FT_3 was lower in females at all ages. Importantly FT_3 declined with age with a clear decrease from 10-14 y, similar but to a lesser degree than the decline reported by Rubenstein et al. (1973). However, due to the use of chronological age and lack of information on maturation status of participants, it is unclear if these alterations in FT_3 appear in relation to pubertal status. In summary, findings support the benefits and requirements for narrower reference ranges, particularly with regard to age around pubertal growth.

2.5.3 Immunoassay & Development of Tandem Mass Spectrometry

Originally diagnosis of thyroid problems was conducted by quantifying circulating TSH using radioimmunoassay, however, this is known to lack sensitivity for a large amount of the population (Jonklaas et al., 2014; Wang & Stapleton, 2010). As such this was superseded by the development of immunometric immunoassays (IA), which are more sensitive yet are not entirely reliable and lack specificity for THs (Wang & Stapleton, 2010). Liquid chromatography-tandem mass spectrometry (LC-MS) has now been considered an alternative method of measuring TH concentrations which has been found to be more accurate, precise, specific and reliable in comparison with IA techniques (Jonklaas et al., 2014; Wang & Stapleton, 2010) in clinical diagnosis as well as for the study of subtle environmental effects on thyroid function (Wang & Stapleton, 2010). Furthermore, sensitivity at high and low concentrations is arguably most important as this is where symptoms and health detriments will occur, and therefore this is where LC-MS could be advantageous. At lower concentrations, between 50-113 mg/dL, LC-MS was found to be more accurate than IA, with correct diagnosis found for 72% and 27% respectively at these low concentrations of T_3. Thyroid hormones measured by LC-MS also showed greater symptomatic agreement than IA (Masika et al., 2016) and therefore LC-MS might be a better reflection of the true clinical situation (Jonklaas et al., 2014). Practically, LC-MS can be conducted using as little as 0.5mL of serum to measure the full TH suite (Wang & Stapleton, 2010). However, the use of non-invasive methods could be beneficial in the monitoring of an individual's thyroid function and be utilised as a preliminary measure to assess if further hormone concentration analysis is required.

2.5.4 Thermogenic Effect of Thyroid Hormones - Body Temperature

With the known limitations and logistical difficulties associated with the diagnosis of thyroid function, and the implied importance of individual reference values (Chatzitomaris et al., 2017; Williams et al., 1978), it is pertinent to find a feasible non-invasive surrogate for thyroid function monitoring, for example, body temperature (BT). Due to the role of THs in mediating BMR, there is a clear relationship with thermogenesis, with many clinical manifestations of thyroid dysfunction relating to this stimulator effect of TH on thermogenesis and energy turnover (Chatzitomaris et al., 2017; Kim, 2008; Silva, 2003; Souetre et al., 1988). It has been suggested that THs largely dictates obligatory thermogenesis (Souetre et al., 1988), however more recent literature has noted dependence on T_3 for facilitative thermogenesis, mainly within BAT (Chatzitomaris et al., 2017; Kim, 2008; Louzada & Carvalho, 2018). Mechanistic reasons for this are proposed to be due to the upregulation of uncoupling protein one (UCP1) in mitochondria of skeletal muscle and BAT in response to T_3 causing an increase in uncoupled oxidative phosphorylation (Chatzitomaris et al., 2017; Kim, 2008; Louzada & Carvalho, 2018; Mullur et al., 2014). As well as this, an absence of THs and catecholamine's significantly reduces the facilitative thermogenesis in BAT, cardiac and skeletal muscle, mediating the uncoupling of ATP hydrolysis from sarcoplasmic calcium cycling and deactivating the sodium and potassium ATPase pump (Kim, 2008; Landsberg, 2012; Silva, 2003). Thus, mitochondrial biogenesis is key for thermogenesis, and THs are in part responsible for modulating all aspects of thermogenesis (Kim, 2008; Louzada & Carvalho, 2018; Silva, 2003).

Due to the documented relationship between THs and thermogenesis, it is understandable that elevated levels of TSH, T_4 and T_3 are seen during colder months in humans if adequate energy is provided to enable this (Chatzitomaris et al., 2017). Conversely, it has been noted that deviations in BT away from the norm can indicate problems with basal thermogenesis and in turn a potential indicator of thyroid dysfunction (Silva, 2003). This theory was discussed as early as 1942 when Broda Barnes suggested the use of a basal BT test as a diagnostic method for thyroid dysfunction (Barnes, 1942), stating that an axillary temperature within 36.6-36.8 ^{o}C indicates healthy thyroid function, and a

variation of 1-2 ºC above or below this is indicative of an overactive or underactive thyroid respectively (Barnes, 1942). Prior research by Du Bois (1921) extrapolated that a 1 ºC increase in rectal temperature is associated with a 10-13% increase in BMR during fever (Landsberg, 2012; Simonsick et al., 2016). Using the mean body mass of adolescent footballers, this reduction in BMR would equate to an approximate increase in BMR of 190 kcal, this is specific practical concern and is meaningful due to the documented changes in BM in paediatric populations with EI changes of 165 kcal/day (Wang et al., 2006).

Few studies have directly assessed TH concentrations and BT fluctuations. One early study in depressed and bi-polar patients reported that, despite circadian variation in both, TSH and BT shared an inverse relationship with a slight phase advancement where a peak in TSH caused a delayed reduction in BT (Souetre et al., 1988). This study had a limited sample size and crucially did not measure circulating TT_3 or FT_3. However, it could be hypothesised that due to low TSH indicating high circulating T_3 levels, a higher BT results when circulating T_3 is elevated. There is a lack of recent direct evidence to support the use of Barnes' basal temperature test to diagnose thyroid dysfunction, however it could offer a practical tool to monitor thyroid function and fluctuations. This could be of a particular benefit to those who could potentially be at risk of acute bouts of supressed thyroid function, such as adolescents under stress (for example, youth athletes), whilst also having the benefit of being appropriate for use in applied settings.

2.5.5 Blood Pressure

In addition to the association between BT and thyroid function, a link between blood pressure (BP) and thyroid function has also been reported. Observational studies have outlined high incidences of arterial hypertension within hyperthyroidism but to a greater extent in chronic hypothyroidism (Fommei & Iervasi, 2002). Early studies directly investigating the impact of THs on BP had conflicting results, with some suggesting hypothyroidism resulted in a lower BP (Endo et al., 1979), possibly due to the related lower pulse pressure (PP), while more thorough investigations noted a significant positive correlation between diastolic blood pressure (DBP) and TH concentrations with hypothyroidism resulting in hypertension in 50-60 y (Saito et al., 1983). However, due to

limitations within study methods, the diagnosis of thyroid dysfunction at the time, and the lack of control for other factors known to impact on BP, the true association between BP and thyroid function as well as underlying pathophysiology in humans remained unclear. This led to Fommei and Iervasi (2002) evaluating the impact of L-T_4 hormone replacement on BP and other associated hormones in 12 patients that had undergone total thyroidectomy. Without T_4 replacement, interpreted as hypothyroid state, systolic blood pressure (SBP) and DBP were significantly increased when compared with L-T_4 hormone replacement. Similarly, "T_4 withdrawal" induced an increase in BP, particularly DBP and the elevation was reversible with L-T_4 treatment, previously reported by Saito (1982). Notably, FT_3 levels significantly negatively correlated with daytime DBP and, the more effectively hypothyroidism was corrected, the lower the resulting DBP, with this significant correlation strongly suggesting a cause-effect relationship (Fommei & Iervasi, 2002).

A large Norwegian population-based study (Asvold et al., 2007) was one of the first studies to evaluate the impact of both overt and subclinical thyroid dysfunction on BP. In over 30,000 individuals, a linear increase in both SBP and DBP was found when TSH increased within reference ranges (0.5-3.5 mU/l), supporting the hypothesis that subclinical hypothyroidism can cause an increase in BP. Notably, although prevalence of hypertension increased with higher TSH concentrations in both sexes, the relationship was stronger in males than females. More specific research into the impact of TSH on BP in children and adolescents was conducted in 12,353 0-17 y olds (Ittermann et al., 2012). A linear increase of SBP and DBP with increasing TSH concentrations was identified within reference ranges, indicating an increased risk of hypertension with subclinical hypothyroidism.

Despite both of these studies evaluating both clinical and subclinical thyroid dysfunction in large cohorts, the reliance on TSH to diagnose thyroid dysfunction has many limitations as previously discussed. A more recent and robust study measured circulating FT_4 and FT_3 in conjunction with TSH concentrations in 880 Chinese school-aged participants without diagnosed thyroid diseases (Chen et al., 2012). In support of the earlier studies (Asvold et al., 2007; Fommei & Iervasi, 2002; Ittermann et al., 2012)

results showed that SBP and DBP was significantly higher in children with subclinical hypothyroidism. After adjustment for BMI, this remained a significant positive correlation for both TSH and importantly FT_3. However, the negative correlation found for FT_4 was non-significant. In further support of Asvold et al (2007), the association was stronger in males than females. In contrast to the specific research into THs on BP in children and adolescents (Chen et al., 2012; Ittermann et al., 2012), Park et al (2016) found no relationship between TSH concentrations and SBP or DBP in 290 euthyroid children. However, FT_4 was found to have a significant negative association with DBP, similar to earlier research (Chen et al., 2012), though after adjustment for BMI, age and sex, this became non-significant. Smaller sample size and lack of assessment of circulating T_3 are major limitations of this study and could explain some of the discrepancies with other studies.

Mechanisms for the increase in SBP and DBP in overt and subclinical hypothyroidism are still unknown, however evaluation of catecholamines in Fammei and Iervasi (2002) study supported suggestions that hypothyroidism is related to stress and that under hypothyroidism conditions, noradrenaline, cortisol and aldosterone were significantly higher than when under $_L$-T_4 treatment. Thus, Fammei and Iervasi (2002) concluded that hypothyroidism induces an increase in BP in conjunction with activation of the sympathetic/adrenal system. However, other mechanisms have also been suggested including the dilation of vascular smooth muscles with T_3 and the relaxation of skeletal muscle arterioles with T_4 (Chen et al., 2012).

Despite some conflicting research, the majority of evidence supports a relationship between BP and THs, with some authors suggesting a cause and effect relationship (Fommei & Iervasi, 2002). Evidence favours elevated BP independent of age and body composition (Brock et al., 2012; Luke et al., 2004), with a significant increase in DBP in hypothyroidism (Luke et al., 2004). This relationship appears to be more consistent in subclinical thyroid dysfunction with a linear relationship between TSH, SBP and DBP. The relationship appears consistently stronger in males. Outside of normal reference ranges, with overt thyroid dysfunction, it appears that both hyper and hypothyroidism can result in increased BP. Logistically, BP could be utilised as an indicator of thyroid

function and used as a practical method of monitoring thyroid function. Furthermore, the link appears to be stronger in males and could be utilised within practical applied settings when invasive measures are not always feasible.

2.6 Lifestyle Factors and Metabolic Rate

2.6.1 Stress and the Stress Reaction

Stress is a term often used to describe an emotional state with a range of physical symptoms. Although the word stress is ambiguous, it is often defined as a state of disharmony or a threat, real or implied, to homeostasis (McEwen & Wingfield, 2003). The basic reaction pattern is consistent irrespective of the agent used to produce stress (Selye, 1946). Therefore, when life is at risk, or homeostasis under threat, the body attempts to combat these threats with a 'fight or flight' response (McEwen & Wingfield, 2003). These responses all require energy greater than those for basic life-sustaining functions and therefore the relationship between stress, the stress-response and energy must be considered (Picard et al., 2018). Subsequently, despite living organisms having an innate ability to undergo adaptation under stress, the action of this can have consequences for health and incur diseases and ailments associated with stress. These can be further exacerbated if the response is inadequate, or if adaptation is chronically required. Therefore, diseases are potentially diseases of the adaptation to stress factors and not stress itself (McEwen & Wingfield, 2003; Selye, 1946).

The basic hormonal stress response is largely mediated by the HPT axis and the autonomic nervous system (Chatzitomaris et al., 2017; McEwen & Wingfield, 2003). An initial suppression of somatotropin, the gonadotrophins and thyrotropin are accompanied by an increased production of adrenocorticotropic hormone (ACTH) that induces an increased production of glucocorticoids, specifically cortisol, adrenaline and TSH (Chrousos & Gold, 1992; Selye, 1946; Sterling, 1988; Tsigos et al., 2016). The resulting acute increase in adrenaline, noradrenaline and cortisol can be considered the body's adaptation to stress in an attempt to survive (Sterling, 1988). However, despite this hormonal stress response having acute protective effects, the chronic adaptive process is believed to have further consequences of its own (McEwen & Wingfield, 2003; Selye, 1946).

Two similar theories exist to support this theory of 'diseases of adaptation'. In 1946 Selye termed this basic reaction response and subsequent illness 'General Adaption Syndrome' (GAS) (Selye, 1946). GAS states that irrespective of source, anything that causes stress endangers life unless it is met by an adequate adaptive response. In turn, any danger to life, or alternation in homeostasis, initiates stress and adaptive responses. Stressors can be physical or emotional, but regardless of source, the adaptive response initiates central and peripheral reactions to preserve homeostasis (Chrousos & Gold, 1992) and thus the illnesses often associated with stress are more 'diseases of adaptation' (Selye, 1946).

In 1988 Sterling and Eyer coined the term allostasis. Allostasis is defined as a dynamic stress reaction built on the principle that, to maintain stability, an organism must maintain all internal milieu within set parameters (Sterling, 1988). There are clear similarities between the theory of allostasis and Selye's GAS theory, with a basis on a physiological stress reaction and fundamentals in homeostatic principles (Selye, 1946). However, Sterling and Eyer (1988) believe that when in a catabolic state, the homeostatic 'set-point' is believed to have greater fluctuation in response to the external factors and concurrent allostatic load (Sterling, 1988) when compared to the GAS. Therefore, the allostatic model defines health as a state of responsiveness, with the internal milieu varying to meet the perceived anticipated demand. In turn, it is ultimately believed that detrimental responses only occur if the external stressors are deemed to be greater than the appropriate allostatic load, and in turn, detrimental consequences are more common when an individual is chronically in a catabolic state (Chatzitomaris et al., 2017; McEwen, 1998; McEwen & Wingfield, 2003; Sterling, 1988).

Similar to the GAS, the allostatic model proposes that it is the response to the stress, or the body's inability to respond effectively, that results in illness and the health detriments associated with stress. Allostasis is outlined as an active process in which the body adapts to stress to maintain bodily functions by continually adjusting stress mediators (immune response, metabolism, cortisol, adrenalin) (Sterling, 1988). The subsequent demand of this allostatic adaptation on the organism is termed the 'allostatic state'. If an individual lacks the resources to adapt to the stress, the allostatic state can become toxic (Picard et

al., 2018). The cumulative impact of this toxic stress on an organism is termed ‘allostatic load’ and can result in chronic physiological dysfunction (Chatzitomaris et al., 2017; McEwen, 1998; McEwen & Wingfield, 2003). If a stress response continues without adequate resources to combat it then ‘allostatic overload’ arises, often when toxic stress occurs in conjunction with health damaging behaviours (Picard et al., 2018). Although GAS and allostasis theories use different terminology to describe the ‘homeostatic set-point’ or ‘allostasis’, they are similar in that it is the body’s adaptation and subsequent demands that could prove detrimental to the HPT axis. Thus, despite a variety of lifestyle factors becoming stressors, the response to stress is uniform (Chatzitomaris et al., 2017; Chrousos & Gold, 1992; Selye, 1946; Walter et al., 2012).

The reproductive, growth and immunity systems are inherently linked to the stress system, each being heavily impacted on by the effects of stress (Chrousos & Gold, 1992). This is often due to the peripheral restraint of these system to preserve energy that can be utilised for the adaptation response to an immediate threat. For example, an acute increase in growth hormone (GH) occurs following a stress response. In contrast, prolonged activation of the stress system results in chronic suppression of GH in man (Chrousos & Gold, 1992). In addition, chronic stress has clear effects on the HPT axis with an association indicated between THs, cortisol and the activation of the sympathetic and adrenal system with hypothyroidism (Chrousos & Gold, 1992; Fommei & Iervasi, 2002; Walter et al., 2012). Alternations in THs in response to stress and the concurrent cortisol increase are rapid, with evidence of T_3 suppression occurring immediately following anaesthesia and remaining for 8 hours, whilst cortisol levels were elevated during abdominal surgery (Michalaki et al., 2001). Furthermore, individuals with hypothyroidism have elevated catecholamines that are reversed with $_L$-T_4 treatment (Fommei & Iervasi, 2002). Despite the exact mechanism being unknown, it is thought that this reflects energy conservation as witnessed in the growth, reproduction and immunity systems. It is believed that the reduction in circulating T_3 is due to the increased levels of glucocorticoids and somatostatin and serves to conserve energy in times of stress (Chatzitomaris et al., 2017; Chrousos & Gold, 1992). In support of this theory, during abdominal surgery in conjunction with increasing cortisol concentration, there was an increase in circulating T_4 and rT_3 whilst T_3 decreased (Michalaki et al., 2001), indicating suppression of metabolic rate and preferential inner ring deionisation to conserve energy.

Notably, the decline in T_3 reported in these studies occurred almost simultaneously with increases in cortisol, independent of IL-6, indicating a potential direct effect of cortisol on T_3.

2.6.2 Stress Factors – Energy Balance

Fasting is known to induce profound metabolic changes in the body to conserve energy through decreased EE (Boelen et al., 2008; van der Spek et al., 2017), potentially due to the selective downregulation of active THs (Chatzitomaris et al., 2017). The exact mechanisms behind this conservation are unknown, but multiple theories from rat and human studies have been proposed (Boelen et al., 2008).

As early as 1973 supressed T_3 concentrations during starvation in obese individuals were reported (Rothenbuchner et al., 1973) and later replicated (Portnay et al., 1974). However, the studies differed, with one noting a decline in T_4 with no increase in TSH (Rothenbuchner et al., 1973), and the other reporting a slight increase in FT_4 (Portnay et al., 1974). Typically, with a reduction in T_4 and T_3 there would be an opposing increase in TSH, however, the lack of this expected finding within the early research suggested that other parts of the HPT axis were involved in the reduced metabolic rate and TH concentrations (Boelen et al., 2008). In support of this, more recent literature suggests that hepatic deiodinase activity and peripheral conversion of THs could also mediate these apparent changes (Chatzitomaris et al., 2017; van der Spek et al., 2017) with fasting concurrently increasing D3 activity and supressing D1 activity (Fliers et al., 2015; van der Spek et al., 2017). These adaptions in deiodinase enzyme activity levels could account for the reported increase in rT_3 after 7 days of fasting which returned to baseline following 4 days of refeeding, alongside the reduction in T_4:T_3 ratio which was apparent within 24 hours (Lopresti et al., 1991). However, the authors commented that the increase in rT_3 was due to decreased clearance and degeneration of T_3 with no significant change in production and therefore supports the rationale for lesser D1 activity as opposed to increased D3 activity. In contrast, the reduction in T_4:T_3 ratio seen in this study (Lopresti et al., 1991), and the delayed increase in rT_3 compared to suppression of T_3 following abdominal surgery (Michalaki et al., 2001), implies a different mechanism impacting on peripheral conversion of T_4, potentially heightened D3 activity to a lesser extent than the

supressed D1 activity. These earlier studies used small sample sizes and both obese and non-obese individuals, which could explain some conflicting findings. They also did not account for EE, thus resulting ED or EA were not reported. The apparent decrease in T_3 with no significant change in T_4 concentration also supports the notion that T_3 and metabolic rate suppression are due to a reduction in hepatic peripheral conversion. Therefore, it could be suggested that during fasting, and under negative EB, there is both downregulation of the HPT axis and altered TH production at peripheral organs resulting in decreased and increased serum T_3 and rT_3 concentrations respectively (Boelen et al., 2008). Therefore, both fasting and energy deprivation result in substantial changes in TH metabolism (Chatzitomaris et al., 2017).

Within athletic populations the term energy availability (EA) is often used. It is defined as the residual energy remaining to support physiological functions after the EE used for exercise (EEE) has been deducted from EI (Burke et al., 2018; Koehler et al., 2016; Loucks & Heath, 1994). Thus, it is often more applicable to refer to EA within sporting populations as opposed to raw EI due to the high EEE. Recently RED-S has become prominent in research and practice. Initially the impact of LEA (≤30 kcal/kg/FFM/day) on health and athletic performance, including the endocrine response, was outlined. However, zones of EA have been derived (see Table 2.4), indicating the impact of different EA on athletic performance and health (Burke et al., 2018; Elliott-Sale et al., 2018; Loucks et al., 2011). With prevalence or RED-S in different sports ranging from 22-58%, there is evidence of an evident problem with LEA in sport (Logue et al., 2020). Research in males, however, is lacking due to the previously acknowledged female athlete triad (FAT) dominating in this field. Research findings are summarised in Table 2.5.

Table 2.4 Classification of EA Zones, Adapted from Loucks et al. (2011)

Energy Availability	**Zone**	**Impact on Health**
>45 kcal/kg FFM/day	High	High EA for growth ↑ BM Adequate energy for all physiological functions
~45 kcal/kg FFM/day	Optimal	Healthy EA for EB ↔ BM Adequate energy for all physiological functions
30-45 kcal/kg FFM/day	Subclinical /reduced	Reduced EA ↓ BM Tolerated for short periods Might impact on some physiological functions
<30 kcal/kg FFM/day	Low	Low EA ↓ BM Insufficient energy for physiological functions Impairment in body systems Reduced training performance and adaptation

FFM, Fat Free Mass; EA, Energy Availability; BM, Body Mass; EB, Energy Balance

The classic work on energy availability and the impact on THs began in 1993 in 46 healthy adolescent females, in which EA was altered through changes in EE whilst EI remained consistent through a clinical liquid diet (Loucks & Callister, 1993). Following 4 days of LEA, TT_3 and FT_3 were reduced by 15 and 18% respectively whilst TT_4 and rT_3 increased by 7 and 24% respectively, suggesting a decline in peripheral conversion of T_4 to T_3 and a preference towards rT_3 production, potentially in an effort to reduce metabolic rate for energy preservation as seen in starvation and fasting studies (Boelen et al., 2008). However, in the energy balanced group, despite the addition of exercise-related stress, adequate EA resulted in T_3 levels remaining similar after four days, implying that low T_3 syndrome was induced by LEA and not due to the acute stress of PA. Thus, the authors boldly claimed that their results unambiguously demonstrate that PA affects thyroid metabolism solely due to its impact on EA. Attempts to identify a threshold at which EA compromise's thyroid function have been undertaken largely by the same research group utilising untrained healthy females (Hilton & Loucks, 2000; Loucks et al.,

1998; Loucks & Heath, 1994). Accordingly, a reduction in TT_3 and FT_3 appears prior to an increase in rT_3, with a reduction in T_3 and FT_3 shown when EA is ≤25 kcal/kgFFM/day and an increase rT3 not apparent until EA is as low as 19 kcal/kgFFM/day. However, this indicates that if EA remains >25 kcal/kgFFM/day in untrained healthy females, the negative implications of PA on thyroid function can be compensated. However, as mentioned earlier, due to individual differences in TH concentrations and narrow population groups, this cannot be extrapolated onto other populations, especially athletic adolescent males.

To date, only two small studies have investigated the effects of LEA and TL on healthy males. One study directly investigated the impact of EA on metabolic biomarkers by controlling for EA with and without exercise in six healthy males (Koehler et al., 2016). Independent of exercise status, LEA resulted in reduced leptin and insulin, however T_3 was not reduced in a LEA state. This could suggest that the alterations in TH concentrations that appear in males following exercise could be more due to the stress impact of exercise on thyroid function in contrast to the EA consequences seen in female subjects. Alternatively, it could be due to the limited EA, 15 kcal/kgFFM/day, not being great enough to evoke negative consequences on TH concentrations despite this being a smaller EA than that reported to have hormonal effects in untrained females. However, due to the lack of research on trained males, and the individual variability in thyroid function, further research is necessary to investigate the impact of LEA on THs in trained males (Logue et al., 2020; Melin et al., 2019). This is further reflected in a study investigating the effects of intensified training on RMR in 13 trained male cyclists (Woods et al., 2018) showing a decrease in RMR during intensive training weeks (140-150% baseline training). Analysis of TL and THs showed no significant effect of TL of FT_3, however the authors noted large individual variability and did indicate altered TH status in response to TL. Therefore, although authors are noting alterations in HPT-axis activity in response to TL and EA in healthy males (Koehler et al., 2016; Woods et al., 2018), the limited sample size combined with known individual variability in thyroid function has resulted in no clear conclusions being drawn but indicating a trend towards suppression in FT_3 when in a LEA state.

Table 2.5 Overview of Studies Assessing the Impact of Energy Availability on Thyroid Hormone Concentrations

Authors	Participants	Energy Availability	Energy Expenditure Through PA	Thyroid Hormone Response
Loucks & Callister (1993)	46 adult females Healthy, sedentary	4 days: 8 kcal/kg/day vs 30 kcal/kg/day	4 days: 0 kcal 1300 kcal at 40% VO_2 1300 kcal at 70% VO_2	↓TT_3 (15%) ↓FT_3 (18%) ↑TT_4 (7%) ↑rT_3 (24%) No impact of exercise intensity or quantity on TH
Loucks & Heath (1994)	27 adult females Healthy, sedentary	4 days: 10.8, 19.0, 25.0 or 40.4 kcal/kg LBM/day	4 days: 30 kcal/kg LBM/day at 70% VO_2	↓ TT_3 (16%) and FT_3 (9%) between 19 and 25 kcal/kg LBM/day ↓FT_4 (11%) and rT_3 (22%) between 10.8 and 19 kcal/kg LBM/day
Loucks, Verdun & Heath (1998)	9 adult females Healthy, sedentary	4 days: 45 or 10 kcal/kg LBM/day	4 days: 30 kcal/kg LBM/day at 70% VO_2	↓TT_3 (18%)
Loucks & Thuma (2003)	29 adult females Healthy, sedentary	5days: 10, 20, 30 or 40 kcal/kg LBM/day	5days: 15k cal/kg LBM/day at 70% VO_2	↓ in T_3 at 30 kcal/kg LBM/day ↓↓ in T_3 at 20 kcal/kg LBM/day
Koehler et al. (2016)	6 adult males Healthy, trained	4 days: 15 or 40 kcal/kg LBM/day	4 days: 15 kcal/kg LBM/day at 60% VO_2	No change in T_3 in any conditions
Heikura et al. (2018)	59 adults Elite endurance walkers	7 day food diary: < or > 30kcal/kg LBM/day		↓T_3 in AME compared to EUM females ↓T_3 in low TES compared to normal TES males Negative correlation between T_3 and injury indices in males

PA, Physical Activity; LBM, lean body mass; AME, amenorrhea; EUM, eumenorrhea; TES, testosterone

2.6.3 Stress Factors - Physical Activity

Exercise classifies as a physical stress, challenging homeostasis and contributing to allostatic load (Lambiase et al., 2012; Mastorakos & Pavlatou, 2005), with PA documented to have impacts on metabolic rate in relation to exercise intensity, duration and training status. Bouts of PA can induce acute episodes of increased metabolic rate, however it has been documented that PA and exercise can supress T_3 concentrations and subsequently reduce metabolic rate up to and beyond 24 hours post PA (Chatzitomaris et al., 2017; Hackney & Saeidi, 2019; Kanaka-Gantenbein, 2005; Lambiase et al., 2012). As explained previously, the energy cost of PA can consequently restrict EA if dietary intake is not adjusted appropriately (Heikura et al., 2017; Koehler et al., 2016), with suggestions that it is the LEA as opposed to the PA stressor that produces negative metabolic alternations (Koehler et al., 2016; Loucks et al., 1998). However, overtraining has been suggested to be a stress-related disorder impacting on metabolic health and thyroid function, with acute aerobic exercise one of the earliest identified factors, along with starvation that results in allostatic responses from the HPT axis (Chatzitomaris et al., 2017; Terjung & Tipton, 1971).

Recent studies have attempted to confirm and quantify the impact of PA bouts (Beyleroglu, 2011; Hackney & Dobridge, 2009; Hackney et al., 2012), exercise intensity (Ciloglu et al., 2005; Hackney et al., 2012) and prolonged training (Hawamdeh et al., 2012; Perseghin et al., 2009) on TH concentrations, all offering comparable results in male trained athletes that are summarised in Table 2.6. With regard to acute bouts of aerobic exercise, both Beyleroglu (2011) and Hackney and Dobridge (2009) outlined a suppression of FT_3 and TSH in the hours following aerobic exercise in male trained athletes. Both studies utilised running until exhaustion in highly trained male athletes and monitored cortisol levels at the same sampling points. Both studies outlined a significant increase in FT_3, TSH and cortisol immediately following exercise, however Beyleroglu (2011) noted a slight suppression in FT_4 at this time point in contrast to the significant increase reported by Hackney and Dobridge (2009). Similarly, at 60 minutes post exercise, cortisol remained heightened and FT_3 and TSH had decreased below baseline in Beylergolu's (2011) study whereas in Hackey and Dobridge's (2009) research FT_3 and TSH did similarly decrease, but only towards baseline, without decreasing beyond this point. Data collection ceased at 60 minutes post-exercise in Beyleroglu's (2011) study but was continued to 90 minutes and 24 hours post-exercise in the earlier study (Hackney

& Dobridge, 2009), giving a greater insight into the endocrine response post-exercise. This led to a key finding that showed a continued suppression of TSH and FT_3 while cortisol remained elevated. Notably, 24 hours after exhaustive exercise, FT_3 and TSH were significantly decreased when compared to baseline, negatively correlating with elevated cortisol. It is important to note that FT_4 remained similar to baseline from 30 minutes post to 24 hours post-exercise (Hackney & Dobridge, 2009) and similarly did not significantly change in male hockey players (Beyleroglu, 2011). These findings indicate that variations in FT_3 concentrations are due to alterations in peripheral conversion of THs (Beyleroglu, 2011; Chatzitomaris et al., 2017), and that deiodination could be inhibited up to, and potentially beyond 24 hours after cessation of PA. As such, if blood sampling protocols are not extended long enough after exercise, TH changes might not be detected and could explain differences in findings (Hackney & Saeidi, 2019). However, due to other metabolites of T_4 (namely rT_3) not being measured in these studies, this cannot be confirmed. A neglected area for both studies is the lack of dietary control. No control or record of nutritional intake was used by Beyleroglu (2011) which must be considered when interpreting the findings. However, despite prescribing a high carbohydrate diet for the 72 hours pre-exercise, Hackney and Dobridge (2009) did not check compliance and crucially did not control intake in the 24 hours following exercise. Increased carbohydrate intake could impact on overall EI as well as liver glycogen stores, and in turn impact on peripheral TH conversion. Furthermore, if EI and carbohydrate is inadequate in the 24 hour period following PA, this could in part be responsible for the supressed FT_3 24 hours after PA, due to the LEA.

Similar, to the findings of Hackney and colleagues, (2009) a recent study of 20 professional male swimmers reported a decline in FT_3 after exercise despite an increase in TSH and FT_4 (Kocahan & Dundar, 2018). Despite the authors interpreting the results based on the concurrent lipid response to TL (an increase in circulating HDL and LDL with increased adrenaline), they did not comment on the potential mechanism behind the variation in THs. Similar to the aforementioned studies, rT_3 was not measured. However it could again be hypothesised that there was a prioritisation of conversion to rT_3 from T_4 in an energy conservation attempt. This is also a possibility as the response became greater with increasing TL, indicating that the greater the TL the greater the stress response and subsequent changes in THs. Although this could be due to EA, EI was not

quantified, and despite an attempt to replicate nutritional intake, this cannot be guaranteed therefore this mechanism is speculative.

In addition to TL, training mode and intensity have also been investigated. A study into 15 well-trained male athletes compared a period of rest to steady state treadmill running at 60-65% VO_{2max} and work rate matched interval running on FT_4, FT_3, rT_3, TSH and cortisol concentrations (Hackney et al., 2012). An increase in FT_4 and cortisol occurred immediately after both exercise conditions compared to the control, with these levels returning close to baseline 12 hours post-exercise. In contrast, despite FT_3 being elevated immediately after both exercise bouts, FT_3 significantly decreased at 12 hours post interval training. Whilst the steady state condition also decreased FT_3 concentrations, the decrease was not statistically significant. In contrast to earlier studies that failed to assess rT_3 concentrations (Baylor & Hackney, 2003; Beyleroglu, 2011; Hackney & Dobridge, 2009; Kocahan & Dundar, 2018), Hackney et al. (2012) showed a negative correlation between FT_3 and rT_3 12 hours after interval exercise.

These findings further support the hypothesis of PA supressing the peripheral conversion of T_4 to T_3 and infer a preference for inner ring deiodination by the D3 enzyme inducing an increase in rT_3 in conjunction with a suppression in T_3. Furthermore, a delayed impact of heightened cortisol on this shift in deiodination is apparent, which could be seen up to 24 hours post-exercise (Hackney & Dobridge, 2009). Higher intensity exercise is also noted to have a greater impact on thyroid function and peripheral conversion of THs (Hackney et al., 2012). A more robust study of 60 well-trained males showed that TSH increased almost linearly with increasing intensity of treadmill running (Ciloglu et al., 2005). However, at lower intensities, FT_3, TT_3, FT_4 and TT_4 all increased when exercise intensity increased from 45% to 70% maximum heart rate (MHR). In contrast, between 70% and 90% MHR a significant decline in TT_3 was noted whilst FT_3 declined slightly and the increase in T_4 and FT_4 slowed. This suggests that at intensities above 70% MHR, the peripheral conversion of T_4 to T_3 could be inhibited, similar to that found in Hackney and colleagues research (2012). However, it must be noted that none of these studies controlled for dietary intake. Therefore, despite being able to dictate the impact of exercise intensity on thyroid function, these overall effects could be induced by a LEA and could be mediated to some extent by balancing EI with the increased EE prompted by the PA bout.

Table 2.6 Overview of Studies Investigating the Acute Impact of Exercise on Thyroid Hormones

Authors	**Participants**	**Exercise Modality**	**Exercise Intensity**	**Nutrition**	**Thyroid Hormone & Catecholamines Response**
Hackney & Dobridge (2009)	22 adult males Endurance trained	Fasted treadmill run to exhaustion	100% VT	Instructed high CHO diet (~60% EI)	**Exercise Cessation :** ↑ TSH, FT_4, FT_3, cortisol **30minutes post exercise:** ↑ Cortisol → TSH. FT_4, FT_3 **24hours post exercise:** ↓ TSH, FT_3, cortisol (sig.) ↓ FT_4 (non sig.)
Ciloglu et al. (2005)	60 adult males Endurance trained	Bicycle ergometer	45% MHR 70% MHR 90% MHR	N/A	TSH, TT_4 FT_4 steady increase with intensity ↓ TT_3, FT_3 at 90%
Beyleroglu (2011)	14 adult males Field hockey players	Outdoor running test to exhaustion	Progressive shuttles	N/A	↓ FT_3, FT_4 and TSH 1hr post-exercise
Hackney et al. (2012)	15 adult males Highly trained	Treadmill running	Interval: 90 seconds at 100-110% VO_{2max}, 90 seconds recovery for 42-47 minutes Steady state: 45 minutes at 60-65% VO_{2max}	N/A	**Exercise Cessation:** ↑ FT_4, FT_3, rT_3, cortisol **12hours post interval:** ↓ FT_4, FT_3 ↑ rT_3, cortisol **12hours post steady:** ↓ FT_4, rT_3 ↑ FT_3, cortisol
Kocahan and Dunbar (2018)	20 adult males Elite swimmers	Indoor swimming	Time trials: 50 m, 200 m, 400 m	N/A	↓ TSH, FT_3 after 400 m ↑ FT_4 after 200 m and 400 m

→, return to baseline; VT, ventilatory threshold; MHR, maximum heart rate; CHO, carbohydrate; TSH, thyroid stimulating hormone; FT_4, thyroxine; FT_3, triiodothyronine; rT_3, reverse-triiodothyronine.

Three notable studies have investigated the more chronic impact of exercise and training on TH concentrations rather than the acute responses summarised in Table 2.7. Two studies identified differences in TH concentrations in adolescent (Hawamdeh et al., 2012) and adult athletes (Perseghin et al., 2009) in comparison to their non-athlete counterparts. Notably, no fasting difference was reported in FT_3 and FT_4 concentrations between the adult athletes and non-athletes (Perseghin et al., 2009). However, TSH was lower in athletes resulting in a lower TSH:FT_3 ratio, accompanied by lower leptin in athletes, supporting the hypothesis that leptin is a mediator in the adaptive thyroidal response in athletes. Due to lack of dietary control within this research, the conflicting findings could be in part due to variations in EA, thus the transient changes in TH concentrations reflecting a hypometabolic state could be counteracted by adequate nutritional intake (Kanaka-Gantenbein, 2005).

With the majority of research focusing on the acute hormonal responses to PA and mostly limited to <24 hour follow ups, longitudinal research is sparse. However, a recent study outlined the variations in endocrine hormones pre and post-season in 16 middle and long distance female athletes (Nicoll et al., 2018). Although no significant differences were found for TSH, T_4 and post season measurements, key findings indicate athletic performance decrements with TH alternations and individual variations supporting a notion for supressed thyroid function following prolonged training. Of particular interest to practitioners, change in T_3 correlated with perception of fatigue in athletes as well as running performance at the end of the season, indicating vital athletic performance concerns associated with thyroid function. Despite statistical significance not being obtained, results suggested large individual variability in baseline TH concentrations as well as in response to training. This could be accounted for by differences in dietary intake. However, a large set of this data was missing. Despite this, the authors did find a trend for lower T_3 values with a lower EI, similar to that in studies outlined in Table 2.5. The mean T_3 value was also in the low end of the reference range whilst T_4 was, by contrast, in the higher range, again indicating a potential for supressed peripheral conversion of THs in athletic populations potentially due to LEA. By contrast, adolescent male athletes were found to have higher TSH and FT_3 than their matched non-athlete counterparts at rest (Hawamdeh et al., 2012). However, without dietary control, it is difficult to compare findings as EA is unknown. These finding give valuable ecological insight into the cumulative effect of training and dietary intake on thyroid function.

However due to the infrequent sampling points, lack of TL data surrounding sampling points, and missing information for dietary intake, it is difficult to interpret the findings fully. Data does suggest suppressed thyroid function following prolonged exercise, especially if combined with inadequate EI resulting in LEA.

Table 2.7 Overview of Studies Investigating the Chronic Effects of Exercise Training on Thyroid Hormones

Authors	**Participants**	**Study aim**	**Thyroid Hormone Response**
Perseghin et al. (2009)	54 adult males 50% Sedentary, 50% endurance trained	Resting TH and RMR in trained vs none trained	↓ TSH:FT_3 ratio in athletes
Hawamdeh et al. (2012)	80 adult male and female athletes 50 sedentary adolescent male and females	Variation in TH in aerobic vs anaerobic athletes	No difference in TSH, FT_4 or FT_3 between aerobic vs anaerobic athletes
		TH in athletes vs sedentary population	↑ TSH and FT_3 higher in athletes ↓ FT_4 in athletes
Nicoll et al. (2017)	16 Female Track and field endurance athletes	Pre and post-season variations in TH	No sig. change in TH across season ↓ FT_3 correlated with ↓athletic performance

TH, thyroid hormones; RMR, resting metabolic rate; TSH, thyroid stimulating hormone; FT_3, triiodothyronine; FT_4, thyroxine

2.7 The Adolescent Footballer

Football (soccer) is characterised as intermittent exercise containing phases of maximal or near-maximal multi-directional efforts (Baptista et al., 2018; Dellal et al., 2010). In senior games, matches typically last 90 minutes played on a 110 x 70 m pitch. However, depending on the age of players, the pitch size and duration can differ, with players categorised as the Youth Development Phase (u12-u16), playing 35 minute halves on a 90 x 60 m pitch for u13, and u14 increasing to 35 minute halves on a pitch 100 x 60 m increasing to 40 minute halves on 110 x 70 m pitch at u15 level. These differences in maturation stage, as well as match format, clearly alter the associated match demands with total distance (TD) covered reported at ~5700 m/h and ~7700 m/h for u11 and u16s respectively, compared to ~11,000 m in the senior men's game (Chmura et al., 2018; Goto et al., 2015). Similarly, differences are noted in other metrics involved with match load including accelerations, decelerations, high-intensity running, sprint distance (SPD) and turns. Importantly there is an apparent age effect with both TD and high intensity distance increasing with age from u11 to u18s (Buchheit et al., 2010; Goto et al., 2015), however positional variations must be noted with central midfielders covering significantly greater distances than central defenders and differences in varying intensities (Baptista et al., 2018; Chmura et al., 2018).

In addition to the physical demands during a competitive fixture, elite adolescent footballers involved within the Premier League academy structure have high TLs, often subjected to a greater weekly TL than adult players (Bowen et al., 2017). With the introduction of the Elite Player Performance Plan (EPPP) in 2011 (The Premier League, 2020) all players involved in the Youth Development Phase (u12-u16) in Category One academies must have at least 10-12 hours of on-pitch contact hours per week. Data from one category one football academy showed an increase in training exposure from 506.67 ± 67.59 to 611.33 ± 77.52 hours/player/year when comparing data from pre and post introduction of EPPP. In contrast, match exposure had a slight decrease but overall total exposure was greater post EPPP introduction (Tears et al., 2018). The u14-u16s squad have been noted to have the greatest total exposure and subsequent load compared to other none senior squads coinciding with rapid variations in growth and maturation, which could be in part responsible for the increased injury occurrence reported within

these squads (Read et al., 2018). Furthermore, outside of formal academy training a large majority of these athletes have other sporting commitments increasing TL and EE with school games, county football and a large majority playing multiple sports. Of relevance to this thesis, internal data indicates an increase in TL with age as reported in other research (Goto et al., 2015; Tears et al., 2018), with increases in injury occurrence within the u13-u16 squads, similar to earlier discussed findings (Read et al., 2018). In addition, anecdotal evidence suggests greatest amounts of training outside of formal academy contact time within the u14-u16 squads, with an increase in school training, county and international commitments, and an increase in additional external strength and conditioning coaching.

2.7.1 Nutrition and Relative Energy Deficiency in Sport (RED-S)

As previously discussed, alternations to the HPT axis occurs amongst systematic illness, exhaustive exercise and in starvation (Rothenbuchner et al., 1973). More recently this has been extended to less severe conditions including energy deprivation (Chatzitomaris et al., 2017), and specifically within athletic populations with LEA (Elliott-Sale et al., 2018). As such, LEA has been found to link numerous health problems in males and females and is now regularly referred to as RED-S (Mountjoy et al., 2014). With specific importance to this thesis, adolescent footballers have previously been reported to be in a significant ED over a competitive week (Briggs et al., 2015a; Russell & Pennock, 2011).

Three studies have been conducted specifically in UK-based adolescent academy level football players (Briggs et al., 2015a; Morton, 2019; Russell & Pennock, 2011). The two earlier studies utilised food diaries and predicted EE. Both reported a substantial energy deficit over the course of a competitive week, with one showing that this was greatest on match and heavy training days (2278 ± 2307 and 2114 ± 2257 kJ respectively), whilst on rest days, EI was not significantly greater than EE (Briggs et al., 2015a). Therefore, despite the known increased workloads and therefore EE within these athletes, there is not an adequate increase in EI, in turn the athlete's nutritional intake could further exacerbate the negative implications of PA on the HPT axis. In contrast a recent study utilising DLW and EI assessed via a mobile application method (Costello et al., 2017) found no significant difference in EI and EE within eight u18 players. However, there

were large individual variations within this study with four participants being in an ED, with two reported at approximately a 1200 kcal deficit (Morton, 2019).

Further to EB, all studies commented that despite meeting protein requirements for recovery, growth and maturation, carbohydrate intake was below current recommendations, mirrored in the insufficient fibre intake (Russell & Pennock, 2011). Despite higher EI and macronutrient intakes reported by Morton (2019) this remained the case and similarly relative carbohydrate intake was greatest in u12 players, significantly greater than that of u18 players. Similar findings have also been documented in academy footballers when assessed via food diary methods (Naughton et al., 2016). This reduced carbohydrate intake has clear implications for football performance but also on hepatic glycogen stores, possibly impairing peripheral conversion of THs and inducing a hypo-metabolic state. Despite the different dietary habits in different countries, research in Spain (Iglesias-Gutiérrez et al., 2005; Ruiz et al., 2005) and France (Leblanc et al., 2002) have similar findings indicating that there is an inadequate carbohydrate intake, often leading to a significant ED and LEA in adolescent male footballers.

The impact of both increasing PA and LEA induced by either increased EE or suboptimal EI has been outlined previously in section 2.7, and the impact of this on THs has been evaluated. Evidence suggests that the low EI that often presents in adolescent footballers might result in supressed THs, namely T_3, potentially at times in which TL is increased. However, the direct implications of EI metabolic health, thyroid function and athletic performance in this population should be examined further.

2.7.2 Maturation

As a player transitions through each stage of the academy structure they are not only developing as a player in technical and tactical ability but also undergoing distinct phases of growth and maturation, including significant changes in body composition (Malina et al., 2017). It is known that these stages of maturation have implications on functional movement, physical performance metrics, injury incidence, position selection and progression to senior football (Malina et al., 2015; Mendez-Villanueva et al., 2011;

Philippaerts et al., 2006; Towlson et al., 2017). However, there might be a lesser reported interaction with thyroid function and T_3 concentrations.

As mentioned in section 2.4.3 there is an abrupt decline in circulating T_3 levels in line with pubertal change (Rubenstein et al., 1973) mirrored in the reduced EE reported within this maturation stage (Mostazir et al., 2016). It could be hypothesised that despite the reported increased energy costs of growth (Millward et al., 1976), the body attempts to reduce other determinants of EE to preserve energy, potentially due to the selective downregulation of active TH (Chatzitomaris et al., 2017). Despite the sudden decline in T_3, this is not accompanied by a reduction in T_4, indicating that there are adaptions in peripheral conversion of THs that could be related to demands on the liver. Within adolescent footballers, this abrupt decline in T_3 occurs simultaneously with increased training demands and periods of peak growth which evidently require greater energy production. Therefore, it is relevant to evaluate the impact of maturation on thyroid function and specifically T_3 concentrations and the combined impact of TL, EA and maturation on thyroid function and how these factors might simultaneously impact on each other.

2.8 Nutrition and Thyroid Function

The pathogenesis of thyroid disorders has links to dietary factors (Larson-Meyer & Gostas, 2020). Early research centred on iodine intakes and goitre in iodine-deplete areas; however this is less common within males in the UK (Vanderpump, 2011). More recently other nutritional risk factors for thyroid function have been evaluated with emerging research into overall energy and carbohydrate intake as well as micronutrients including selenium, iron and vitamin D (Larson-Meyer & Gostas, 2020). Selenium acts as a cofactor to the deiodinase reactions and is imperative to peripheral conversion of THs. As such, low intakes could have a detrimental effect on TH metabolism and T_3 concentrations and is associated with an increased risk of Graves' disease (Ventura et al., 2017; Zheng et al., 2018). Evidence from human studies on selenium supplementation is conflicting and inconclusive showing varying changes in THs with improvements in selenium concentrations. The most promising findings come from those with severe deficiencies. It is proposed that the optimum range for selenium concentrations is narrow, and due to

the potential toxicity of selenium, supplementation should be considered with caution (Winther et al., 2020). When compared to sedentary individuals, athletes have been found to have lower selenium concentrations, decreasing further during exhaustive exercise (Maynar et al., 2018), potentially due to the greater selenium requirements necessary with increased EE (Margaritis et al., 2005). However, the relationship between selenium concentration, intakes and TH concentrations in athletes remains unknown.

The impact of iron deficiency is well documented within athletes with estimated prevalence reported at ~ 15–35% of female and ~ 3–11% of male athletes (Sim et al., 2019). Prevalence of iron deficiency is thought to be higher among adolescent athletes due to the additional strain of exercise, maturation, foot strike haemolysis, excess loss in sweat, urine or faeces and inadequate EI, with consequences for bone health, fatigue, muscle function and work capacity (Lukaski, 2004; Shoemaker et al., 2020). With regard to thyroid function, iron deficiency can result in decreased T_4 and T_3 (Ashraf et al., 2017; Hess, 2010; Luo et al., 2017). The mechanism responsible for this is unknown and could be due to impaired thyroid peroxidase activity slowing coupling of MIT and T_2 (Ashraf et al., 2017; Larson-Meyer & Gostas, 2020). Studies assessing thyroid function and iron supplementation in athletes are limited. One case study in two iron-deficient-anaemic female athletes outlined increased TSH and decreased T_4 with iron supplementation (Rosenzweig & Volpe, 2000). However, EI and vitamin C consumption was not controlled and decreased dramatically in one participant which could have substantially impacted on the findings. Although iron deficiency is apparent in athletes, and there are implications for health and athletic performance, the association with thyroid function and iron intake or deficiency is less clear.

Vitamin D is becoming more widely researched within athletic populations, largely for its role in musculoskeletal health (de la Puente Yagüe et al., 2020). With regard to the thyroid, vitamin D is also recognised as a regulator of inflammation and immune modulation; as such it has been suggested that Vitamin D deficiencies could be a risk factor in AITD, with low serum levels of 25-hydroxycholecalciferol related to AITD in both adults and children (Kim, 2017; Wang et al., 2015). Supplementation with Vitamin D has been assessed in a two recent studies; both showing no significant difference in

TSH or FT_4 following supplementation periods of 4 months at 1200-1400 IU/day (Mazokopakis et al., 2015), and 3 months at 60,000 IU/week (Simsek et al., 2016). In both studies, participants had AITD but were on L-thyroxine treatment so classified as euthyroid, which could explain the lack of change in TH concentrations. Despite the prominence of vitamin D insufficiency in athletes, particularly within the UK (Farrokhyar et al., 2015), to date no studies have directly assessed TH and vitamin D intakes or supplementation in athletes.

In athletic populations it could be proposed that EI and macronutrient composition could play a large role in thyroid health, with noted existing problems within these areas. RED-S has established an association between LEA and endocrine dysfunction, with specific reference to supressed T_3 (Mountjoy et al., 2014), previously documented in fasting research (Boelen et al., 2008). Furthermore, concerns surrounding carbohydrate intake have been evaluated within adolescent footballers (Briggs et al., 2015a; Morton, 2019; Russell & Pennock, 2011) and interventions to rapidly replenish muscle and liver glycogen have been evaluated (Fuchs et al., 2016a; Gonzalez et al., 2017a). Although there is evidence to suggest benefits of selenium, iodine, iron and vitamin D supplementation for thyroid health, it is unknown if this would benefit adolescent athletes. Despite some known deficiencies having high prevalence within adolescent athletes, including Vitamin D and iron, the relationship between these and THs is inconclusive and debate surrounding supplementation protocols remains ongoing. As such there could be more beneficial ways to improve thyroid function in adolescent athletes, possibly through ensuring adequate EA and carbohydrate availability.

2.8.1 Energy Balance

As discussed, there are well documented cases of supressed thyroid function, specifically a decline in T_3, during periods of starvation, fasting, and during bouts of LEA (Boelen et al., 2008; Fliers et al., 2015; Loucks et al., 1998; Loucks & Callister, 1993; Loucks & Thuma, 2003). In some cases, this decline is observed within 24 hours of the induced ED (Lopresti et al., 1991). However in others, suppressed TH concentrations are documented after more sustained ED (Heikura et al., 2017). This could be due to the severity of the ED or other confounding factors such as increased PA, specifically from high intensity

activity (Hackney et al., 2012) and heightened catecholamines due to increased stress factors including military training and cold exposure (Landsberg, 2012). To date, there has been no attempt to outline the ED required to evoke a suppression in T_3 in males, however in untrained females this has been documented when EA is ≤25 kcal/kg/FFM/day (Hilton & Loucks, 2000; Loucks et al., 1998; Loucks & Heath, 1994). Similarly, neither the latency period between an induced ED and suppressed T_3 nor the duration of ED required to induce T_3 suppression have been identified

In adolescent footballers, notable EDs have been documented over the course of an in-season week, with reported mean ED as great as 1302±1662 kJ when assessed by weighed food diaries, food recalls and predictive EE calculations (Briggs et al., 2015a). In contrast, preliminary findings indicate no significant ED in u15 and u18 squads across an in-season training week when assessed by DLW; however sample size (n=8) and large variation must be considered, with two u18 participants having an ED of approximately 1200 and 450 kcal, similar to those reported by Briggs et al. (2015a) (Morton, 2019). Of particular importance is the large deficits evidenced on match days 2278±2307 kJ (Briggs et al., 2015a), which has previously shown to evoke a decline in FT_3 in females (Loucks & Heath, 1994; Loucks & Thuma, 2003). Therefore, it could be postulated that this similarly could result in lowered circulating T_3 within adolescent male footballers. As such, it is relevant to identify if rectifying this ED would attenuate the decline in circulating T_3.

Notable gaps in knowledge exist around periodisation of training and nutrition, with preliminary data showing that the magnitude of within-day ED can alter physiological outcomes (Burke et al., 2018). Additionally, in elite male cyclists, despite daily EB remaining consistent, a greater number of hours in ED (>400kcal) or larger single-hour ED resulted in suppression of RMR, increased cortisol and reduced testosterone (Torstveit et al., 2018). During a standard training week in footballers, the largest TL is typically on a match day (MD), with other high load training days often ‘loaded’ towards the start of the training week, for example on a Tuesday (MD-4) when TD, high speed running (HSR) and SPD are greatest (Akenhead & Nassis, 2016). As such, similar ED could exist on these days. However it must be understood that this can vary dependant on

the fixture played, individual player characteristics, playing style and team characteristics (Di Salvo et al., 2009).

The EE associated with match play is largely out of the player's control and outside of the parameters of their playing position, therefore it is the responsibility of the player to adjust EI accordingly to maintain EB and minimise the potential impacts of an ED. As with many sports, on MDs the eating opportunities are often more limited than normal, specifically during away fixtures when travel can further disrupt a player's nutritional habits. This could in part be responsible for the substantial ED. Therefore, these factors must be considered when implementing nutritional strategies and presented to players as preferential logistically viable options.

2.8.2 Carbohydrate

The impact of carbohydrate intake on sports performance and health is well evidenced (Burke et al., 2011). Consequently, the impact of carbohydrate intake on overall EI and EA is clear, with ED often occurring due to carbohydrate intakes below recommendations in a range of sports including in adolescent football players. Four studies have directly assessed nutritional intake in young professional footballers in the UK. Morton (2019) noted a significant difference of carbohydrate intake with age, with u12s consuming 7.3±0.1g/kg compared to 5.8±0.8 g/kg and 4.8±0.6 g/kg seen in u15 and u18 respectively, similar to those reported by (Naughton et al., 2016). This supports other research similar to those reported in u18s, 5.9±0.4 g/kg (Russell & Pennock, 2011), and u16 players, 5.6±0.4 g/kg (Briggs et al., 2015a). As such, all reported values are lower than those proposed to support athletic performance of 5-7 g/kg and 7-10 g/kg for moderate and intensive training respectively (Burke et al., 2006; Desbrow et al., 2014). In addition to the clear impact this can have on sports performance and recovery and likelihood of LEA, low carbohydrate availability itself can also be an additional stress factor to ED and bone health as seen in elite male and female cyclists (Viner et al., 2015). Furthermore, in sedentary females when LEA was induced but carbohydrate status preserved due to less oxidation, the hormonal impairments usually seen in LEA, specifically LH, were attenuated (Loucks et al., 1998). Similarly, two studies found that when epileptic patients received a ketogenic diet there were significant reductions in T_3 alongside significant

increases in TSH, T_4 and rT_3,though EI was not quantified in either study (Kose et al., 2017; Molteberg et al., 2020). Consequently, it could be hypothesised that during periods of LEA if carbohydrate status is adequate the hormonal impact could be lessened, however this has not been directly assessed in adolescent footballers or specifically with THs.

Although unknown, it has been hypothesised that the impact of fasting and low carbohydrate availability on TH is related to liver glycogen stores, as depleted liver glycogen stores could result in reduced T_3. Accordingly, it could be advantageous to ensure adequate hepatic glycogen stores, even during periods of LEA, and utilise strategies to rapidly replenish liver glycogen following glycogen depletion. Carbohydrates differ in rates of digestion, absorption, chain length, transport proteins and hepatic metabolism (Table 2.8). As such, carbohydrate delivery to the liver can be a rate limiting factor in hepatic glycogen resyntheses. Therefore, exogeneous carbohydrate type must be considered when implementing a nutritional strategy to promote rapid glycogen replenishment. With specific reference to hepatic glycogen stores, in contrast to muscle, the liver can synthesise glucose and resynthesise liver glycogen directly from glucose or from 3-carbon precursors including fructose, galactose, pyruvate and lactate (Gonzalez et al., 2016). As such, fructose and glucose co-ingestion (sucrose) could more rapidly replenish liver glycogen than glucose alone.

Table 2.8 Common Dietary Carbohydrates and Major Intestinal Transport Proteins

Carbohydrate	Chain Length	Constituent Monomers	Bonds	Membrane Intestinal Transport Proteins
Glucose	1	-		**SGLT1**; GLUT2; GLUT 12
Fructose	1	-		**GLUT5**; GLUT2; GLUT7; GLUT8; GLUT12
Galactose	1	-		**SGLT1**; GLUT2
Maltose	2	Glucose + Glucose	α-1,4-glycosidic	**SGLT1**; GLUT2; GLUT8; GLUT12
Sucrose	2	Glucose + Fructose	α-1,2-glycosidic	**SGLT1; GLUT5;** GLUT2; GLUT7; GLUT8; GLUT12
Isomaltulose	2	Glucose + Fructose	α-1,6-glycosidic	**SGLT1; GLUT5**; GLUT2; GLUT7; GLUT8; GLUT12
Lactose	2	Glucose + Galactose	β-1,4-glycosidic	**SGLT1;** GLUT2; GLUT12

GLUT, glucose transporter; SGLT, sodium-dependent glucose transporter. Major transport proteins highlighted in bold. Adapted from Gonzalez et al. (2017)

To date three studies have directly assessed the impact of dietary carbohydrates on tissue specific glycogen replenishment using ^{13}C magnetic resonance spectroscopy, following glycogen depletion in trained males (Casey et al., 2000; Decombaz et al., 2011; Fuchs et al., 2016). All three studies have focused on endurance trained athletes, due to the impact of endogenous glycogen stores on endurance performance, and compared glucose and fructose co-ingestion (sucrose) with glucose. All studies indicated more rapid replenishment of hepatic glycogen stores and liver volume with sucrose than compared to glucose. However, the effect is clearest when carbohydrate ingestion rate exceeds 0.9 g/kg/h (Gonzalez et al., 2017), with significant differences found when 1.5 g/kg/h was consumed for 5 hrs post-exercise (Fuchs et al., 2016) and 1.15 g/kg/h over 6.5 hrs (Decombaz et al., 2011).

The magnitude of liver glycogen depletion used within these studies was intended to near fully deplete stores, resulting in post-exercise liver glycogen stores of 116-191 mmol/l across studies (Casey; Decombaz; Fuchs). Although no study to date has directly assessed the impact of football match play or training on liver glycogen depletion, it is unlikely to be as extreme as those previously outlined. However, in the absence of endogenous carbohydrates, liver glycogen stores can be depleted by ~40%–60% within 90 min of moderate- to high-intensity (~70% VO_2 peak) exercise, potentially more reflective of football match-play and training. Furthermore, match play exhibits periods of higher intensities including HSR, sprinting, jumping, physical contacts, accelerations and decelerations, which are associated with higher rates of liver glycogen utilisation and in turn depletion (Gonzalez et al., 2017).

Although the extent of liver glycogen depletion in adolescent footballers remains unknown, it is known that they are frequently in severe ED with low carbohydrate intakes, including on MD-1 and MD, with training often existing on MD+1. Accordingly, it could be beneficial to implement nutritional strategies to restore liver glycogen as rapidly as possible. In conjunction, there is evidence to suggest adequate carbohydrate intake can positively influence THs and T_3 which could be at risk with heightened cortisol or during LEA following heavy training and match play.

2.9 Summary

The majority of the established thyroid research is clinical, evaluating diseases, prevalence and function. Only over recent decades have disturbances in thyroid function outside of clinical diagnosed conditions begun to be discussed. As such, methods of assessment have been critiqued due to their inability to be conducted in applied settings or account for circadian fluctuations and individual variation in THs. Within athletic populations this is problematic as, although it is suggested that exercise and ED can cause decrements in T_3, there are no valid field-based methods to assess this. This is of particular importance due to the prevalence of RED-S syndrome in a range of athletic populations, including the impact of LEA on thyroid function. Despite this there remains a lack of direct applied research into the impact of athletic performance, nutrition and lifestyle on thyroid function

The acute and chronic effects of stress on thyroid function are well evidenced within the literature. Within athletic populations, specific interest is in EA and the impact of limited EI or increased EE on thyroid function, culminating in the RED-S syndrome. As such, the general consensus within athletic populations is that it is the ED not the stress of exercise that is negatively impacting on thyroid function. Accordingly, by ensuring adequate EA, it could be postulated that any disturbances in thyroid function can be attenuated. Within adolescent football, a greater understanding of the physical demands indicates periods of high TL fuelled with inadequate nutrition often coinciding with players undergoing maturation. As an abrupt decline in T_3 can be seen around the onset of maturation, these combined factors could place adolescent footballers at risk of thyroid dysfunction with implications for health and athletic performance. However, as it is hypothesised to be the LEA causing the largest decrement in T_3 it could be postulated that ensuring adequate EI and carbohydrate intake could preserve thyroid function.

2.9.1 Study Aims

The overarching aim of this thesis is to investigate the impact of maturation, TL and nutritional intake on thyroid function in elite adolescent footballers. The central hypothesis is that T_3 will be supressed when multiple stress factors appear concurrently (including maturation and an induced ED due to increase EE and inadequate EI). This is thought to be due to impaired peripheral conversion of TH. As such, T_4 might remain stable though T_3 is supressed. Subsequently, it is hoped that carbohydrate feeding might improve TH deionisation and preserve T_3 levels in these conditions. This review of the literature has identified multiple areas which require further study as well as implications for working and researching within the applied setting. These will be addressed in the subsequent experimental chapters by raising the following questions and assessed as described below:

1. How can we logistically and reliably assess thyroid function in the sports environment?
2. Is thyroid function supressed in adolescent footballers?
3. What risk factors are associated with supressed thyroid function in adolescent footballers?

4. How can we intervene nutritionally to preserve thyroid function in adolescent footballers?
5. When should we intervene to preserve thyroid function in adolescent footballers?

Study One and Two: Methods of Assessing Thyroid Function in Adolescent Athletes

Aim: Develop logistically viable and reliable methods to assess thyroid function of adolescent footballers in the applied environment.

Objective One*: To validate digital methods of BP and BT assessment*

Objective Two*: To evaluate the reliability of LC-MS analysis of THs in adolescent males*

Objective Three: *To evaluate the reliability of digital methods of BP and BT assessment in adolescent males*

Objective Four*: To assess the relationship between BP, BT and THs in adolescent males*

Study Three: The Impact of Training Loads and Nutritional Intake on Thyroid Function in Adolescent Male Footballers

Aim: Examine the relationship between maturation, TL, EE and EI on thyroid function in adolescent male footballers to identify periods in which thyroid function might be at risk and outline the risk factors associated with suppressed thyroid function.

Hypotheses:

a) The suppression of THs is most likely at times of induced ED, this is most likely on match days when EE is greatest
b) The suppression of THs will be more significant in individuals undergoing maturation
c) The suppression of THs is likely to appear due to alterations in peripheral conversion, as such it might appear as supressed T_3 but with no concurrent decline in T_4

Objective One: *Evaluate when thyroid function is supressed in adolescent footballers*

Objective Two: *Outline and evaluate potential risk factors for thyroid function in adolescent footballers*

Objective Three*: Assess how different THs (T_4, T_3, rT_3 and T_2) respond to stress in adolescent footballers*

Study Four: The Acute Impact of Glucose and Fructose Co-Ingestion on Thyroid Function in Adolescent Male Footballers

Aim: Investigate the efficacy of the acute impact of glucose and fructose on attenuating the impact of stress on THs during identified periods where thyroid function is supressed.

Hypothesis: The co-ingestion of glucose and fructose will restore T_3 levels back to individual set-points following a bout of strenuous exercise.

Objective One*: Investigate how glucose and fructose co-ingestion impacts on THs concentrations*

Objective Two*: Evaluate the impact of the intervention on daily EI and EB*

Chapter 3:
General Methods

3.1 Introduction

The methods described below are those utilised frequently in investigations throughout this thesis. Those unique to specific studies are not included within this chapter and are instead outlined within the relevant chapters. Complete study deigns are described in detail within each chapter with accompanying schematics where appropriate. Institutional ethical approval was granted prior to data collection for all investigations. In addition, written informed consent was sought prior to study commencement (Appendix 1) following a verbal and written briefing relevant to the population group (Appendix 2). This research programme was conducted in collaboration with Newcastle United Football Club (NUFC), as such, to maintain ethical and professional standards written project approval was sought from the Academy Manager prior to the commencement of any research.

3.2 Biological Age and Anthropometric Assessment

Anthropometric data was measured according to ISAK procedures by a Level One Anthropometrist including stretch stature to the nearest 0.01 m using a Harpenden Portable Stadiometer (Holtain Limited, Pembs, UK) and BM measured to the nearest 0.1 kg using portable SECA scales (SECA, UK) to calculate BMI (kg/m^2). In addition, seated stature was measured to the nearest 0.01 m using a Harpenden Portable Stadiometer (Holtain Limited, Pembs, UK), and somatic assessment data was input into Equation 3.1 to calculate age from PHV (y) as a measure of maturation status and biological age (Mirwald et al., 2002). From the calculated maturity offset participants were classified into one of three categories according to their maturation status indicated in Table 3.1, with circa-PHV reflecting the period of most rapid growth for adolescents.

Equation 3.1: *Maturity Offset = -9.236 + (0.0002708 x Leg Length x Sitting Height) + (-0.001663 x Age x Leg Length) + (0.007216 x Age x Sitting Height) + (0.02292 x Weight by Height Ratio)*

Table 3.1 Maturation Status Calculated from Maturity Offset Equation (Mirwald et al., 2002)

Maturity Offset (y)	Maturation Status
-3 to < -1	Pre-PHV
-1 to +1	Circa-PHV
>1	Post-PHV

PHV, Peak Height Velocity

The specific population within this research undergo frequent periodic maturity assessment using this method, approximately every 6-8 weeks, therefore improving the applicability of the maturation assessment utilised. However, due to the error associated with the predictive equation in males ($r^2 = 0.89$, SEE = 0.59 y) it must be considered when interpreting findings that at times individuals might be misclassified into the incorrect group (Hammami et al., 2016; Mirwald et al., 2002).

In addition to maturation assessment, anthropometric assessment of athletes allowed for prediction of body composition. Within the adolescent male footballers' logistical issues prevented the use of more 'gold-standard' anthropometric assessment such as dual-energy X-ray absorptiometry (DXA), however, concerning the aims of this thesis and as body composition was not an outcome measure, alternative methods could be utilised to control for body composition. Within adolescent male footballers the use of four site skinfold (triceps, abdominal, anterior thigh and medial calf) are frequently used and show strong correlation with DXA when the predictive equation below (Equation 3.2) is employed ($r^2 = 0.88$) (Reilly et al., 2009). Therefore, the four-site skinfold method is employed periodically within the athletes participating in the study and was utilised within this research to assess body composition.

Equation 3.2: *Body fat (%) = 5.174 + (0.124 x thigh) + (0.147 x abdominal) + (0.196 x triceps) + (0.130 x calf)*

3.3 Axillary Body Temperature

In all studies axillary BT was only assessed via digital thermometer (Omron Corporation, Tokyo, Japan) with exception to Chapter 4 in which this method was validated against a

mercury thermometer (Oral Association Medical M684525, New York). In all studies, the thermometer was placed high into the participant's left axillary region for two minutes whilst participants sat upright in an ambient temperature room (21.70 ± 0.37 ^{o}C). Following a period of two minutes the reading displayed on screen was recorded. Unless not feasible, this was measured and recorded by the principal researcher, otherwise, participants were responsible for recording their own BT. If this occurred, it was previously ensured that participants were capable of accurately assessing their own BT following verbal and visual instructions. Timing of BT assessment reflected the standard length of football matches at the relevant age groups, however due to the nature of study designs and logistical issues, slight variances exist. Specific timings and study designs are outlined in relevant chapters.

3.4 Blood Pressure

In all studies BP was assessed only via a digital wrist-based sphygmomanometer (Firhealth, China), except for Chapter 4 in which this method was validated against the manual mercury sphygmomanometer method (Accoson, UK). In all studies, the digital sphygmomanometer was placed on the participants left wrist ~250 mm from the distal radial head that was resting on a horizontal surface level with the heart. Following 10 minutes of rest, the device was activated, and BP measurement was recorded as displayed on screen. This same method was replicated a further two times with a two minute period of rest between each measurement, with the mean recorded as the participants BP (Pickering et al., 2005). As with BT, timing of BP assessment reflected the length of football matches at the relevant age groups however due to the nature of study designs and logistical issues slight variances exist, specific timings and study design are outlined within relevant chapters.

3.5 Blood Sampling and Analysis

In all studies, excluding Study One, approximately 500 µl of whole blood was collected via fingertip-capillary sampling (providing ~200-300 µl of plasma for subsequent analysis) to allow for the quantification of THs; FT_4, FT_3, rT_3 and T_2. Prior to sample collection, participants' hands were submerged in warm water for three minutes to enable

more efficient sampling, and fingertip-capillary blood was obtained from a pre-warmed fingertip pierced with a sterile automated lancet (Accu-Check, Mannheim, Germany) whilst participants were sat upright. Blood samples were drawn into pre-cooled EDTA-treated microvettes that were centrifuged immediately for 10 minutes at 5000 rpm. Plasma samples were maintained and transported between 2-8 ^{o}C on each day of testing before being stored at -80 ^{o}C prior to analysis

3.5.1 Analysis

Samples were prepared by transferring 10 µl of sample to microcentrifuge tubes and adding 10 µL of deuterated T_4 (internal standard) and 980 µl of 0.1% formic acid in water, before being centrifuged at 5000 rpm for five minutes. The supernatants were loaded onto a HyperSep™ C18 solid phase extraction (SPE) cartridge, that were preconditioned sequentially with 2 ml of 0.1% formic acid in methanol and 2 ml 0.1% formic acid in water. The cartridge was washed with 1 ml of 30% methanol in water and then the target compounds were eluted with 1 ml of 80% methanol in water. The eluent was dried down under a stream of nitrogen and reconstituted in 50 µl of mobile phase. As the internal standard was a deuterated form of T_4 and therefore structurally the same as T_4 this accounted for interaction during extraction and injection with the different molecular weight and allowed for identification by mass spectrometry. Standards of T_4, deuterated T_4, T_3, rT_3 and T_2 were purchased from Sigma Aldrich (Poole, Dorset) with a purity of ≥98%. Organic LC-MS grade solvents; methanol and acetonitrile, acetic acid and formic acid were purchased from Sigma Aldrich (Poole, Dorset). Hypersep™ C18 100 mg solid phase extraction (SPE) cartridges were purchased from Thermo Fisher Scientific (Hemel Hempstead, UK). Nylon 0.2 µm syringe filters were purchased from Thames Restek (High Wycombe, UK).

3.5.2 Instrumentation

For LC-MS analysis, chromatographic separation was achieved using a Thermo Surveyor LC (Thermo Scientific, Hemel Hempsted, UK) consisting of a quaternary MS pump, vacuum degasser, a thermostated autosampler (set to 5 °C) and a thermostated column oven (set to 25 °C). Mass Spectrometry was performed using a LTQ XL ion trap mass spectrometer (Thermo Scientific, Hemel Hempsted, UK) equipped with a heated

electrospray ionisation (HESI) source. Instrument parameters are shown in Table 3.2. The mass spectrometer was operated in SRM MS/MS in negative mode. Chromatographic separation was achieved on a reversed phase pentafluorophenyl column (Supelco 2.1 µm F5, 100 x 2.1 mm) purchased from Sigma Aldrich. Sample aliquots of 10 µl were introduced onto the column at a flow rate of 200 µl/min.

Table 3.2 Parameters for LC-MS Analysis of Thyroid Hormones (FT4, FT3, rT3. T2)

Parameter	Condition
Flow Rate	200 µl/min
Mobile Phase A	10 mm ammonium acetate in water (pH 4.0)
Mobile Phase B	10 mm ammonium acetate in methanol (pH 4.0)
Isocratic Composition	30%A 70%B
Run Time	8 minutes
Column	Ascentis Express F5 15cmx2.1mm
Particle Size	2.7 µm
Scan Type	SRM MS/MS
Polarity	Negative
Sheath Gas Flow Rate	15 (aux)
Aux Gas Flow Rate	60 (aux)
Sweep Gas Flow Rate	1 (aux)
Spray Voltage	3.5 kV
Capillary Temperature	320 °C

SRM, Selected reaction monitoring

3.6 Nutritional Intake

In Study Three and Four nutritional intake was assessed using similar methods, with specific details outlined within the relevant chapters. In both studies, nutritional intake was assessed throughout the duration in free-living conditions using the combined methods of self-reported weighed food diaries supplemented with 24-hour food recalls, using the two-pass method. This method has been found to have good agreement for EI when compared to observed intake in male academy footballer (CV = 3.1 %) (Briggs et al., 2015b). With an appropriate adjustment equation (Equation 3.3) to account for

consistent statistically significant under-reporting within adolescent male footballers this method can be utilised for determining EI and has such been used in studies assessing potential ED within this population (Briggs et al., 2015a).

***Equation 3.3**: y = 1.0397x - 0.1064*

y = predicted adjusted energy intake; x = participants self-reported energy intake

Prior to the commencement of any studies involving dietary analysis, participants and parents were verbally and visually instructed on how to complete a food diary using the templates provided. Participants completed their food diaries during the appropriate time for each study and underwent a brief food recall every 24 hours. The food recall used the two-pass method (Ashley & Bove, 2003) and was used to cross-reference with the previous 24 hours of recorded food intake to supplement any missing information. Commercially available software was used to analyse nutritional intake (Nutritics v5.097, Dublin), with the principal investigator responsible for all dietary analysis to ensure consistency.

3.7 Energy Expenditure

In Study Three and Four EE was assessed over differing time periods based on the purpose and design of the study. Within both studies EE was calculated in both free-living and exercise conditions, both are explained below with specific study characteristics outlined within the relevant chapters.

3.7.1 Resting Metabolic Rate

Assessment of RMR was undertaken via predictive equations in all studies as assessment via indirect-calorimetry was unfeasible and impractical within the applied environment. Furthermore, due to not being able to accurately assess fat free mass (FFM) via DXA in all participants predictive equations that used BM and stature as variables were used to standardise across studies. Within Study One and Two participants were not academy standard footballers and ,as such, equations derived from non-athletic populations were

deemed appropriate, as such (Henry, 2005). Within Study Three and Four, as all participants were Category One English Premier League soccer academy players, a novel equation developed within this population was utilised (Hannon et al., 2020).

3.7.2 Free Living

Free living EE was assessed using accelerometry methods (ActiGraph, GT3X+, Actigraph, Pensocola, Flordia, USA) which have demonstrated valid and reliable measures of PA levels and sedentary time in adolescent and youth populations (Evenson et al., 2008; Hänggi et al., 2013; Puyau et al., 2004; Santos-Lozano et al., 2013; Trost et al., 2011). Despite attempts to ensure consistent instrument use for each participant in each study logistically this was not possible, however these devices demonstrate high inter-instrument reliability (ICC = 0.97 to 1.00) (Jarrett et al., 2015). Accelerometers were provided to participants for the duration of each test condition, participants were visually informed on the correct placement of the accelerometer and indicated comprehension of this to the principal researcher. Participants were instructed to wear the accelerometer at all times during each data collection bout except exposure to water-based activities. However, some participants removed the accelerometer during sleep-time due to discomfort.

Accelerometer positioning and outputs reflected manufacturers' instructions and previous calibration studies within similar population ages (Evenson et al., 2008; Reilly et al., 2008; Trost et al., 2011). The accelerometer was positioned above the anterior spine of the iliac crest in line with the anterior axillary line of the dominant hip as per the manufacturer's recommendations (Reilly et al., 2008). Raw accelerometer data was analysed using ActiLife 6 Software (Actigraph, Pensacola, Florida, USA) with a 12-bit analogue-to-digital convertor at a user specific rate of 30 Hz digitised the acceleration output. This allowed for more accurate classification between moderate and vigorous PA, when compared to 60 Hz, whilst still accounting for sporadic movement patterns associated with children (Reilly et al., 2008). Metabolic equivalent task (MET) thresholds used are outlined in Table 3.3 based on prior calibration research (Freedson, 2005) with cut-points devised by Evenson et al. (2008) with the Freedson VM3 Combination equation (Freedson, 2005) used to convert raw data to a biological construct of EE.

Although MET values can be used within some research domains due to the nature of this thesis the assessment of EE compared to EI was required, therefore data is expressed as kJ and kcal to allow direct comparison. As such, an EE calculation was required from daily MET values derived from the raw data. The equation utilised is shown in Equation 3.4 adapted from Ridley et al. (2008), using the equation from Schofield (1985) (Equation 3.5) to predict RMR, and has since been used in similar studies assessing energy demands of academy footballers (Briggs et al., 2015a).

Table 3.3 MET Intensity Threshold and Cut Points

	MET Intensity Thresholds (METs)	Cut Points (Activity Counts)
Sedentary Activity (SED)	<1.5	≤100
Light Physical Activity (LPA)	≥1.5 and <4	>100 and <2296
Moderate Physical Activity (MPA)	≥4 and <6	≥2296 and <4012
Vigorous Physical Activity (VPA)	≥6	≥4012

MET, Metabolic Equivalent

***Equation 3.4**: Energy Expenditure (MJ) = MET value × adolescent RMR (MJ·kg−1·min−1) × kg body weight × number of minutes activity performed*

***Equation 3.5**: Resting Metabolic Rate (RMR) = 17.686 × body weight(kg) + 658.2*

3.7.3 Exercise Conditions

In studies Three and Four EE was calculated during structured formal academy-based training. This was quantified based on TL calculated ideally using metrics from a GPS, however if this was not possible due to logistical issues and cost of units then session rating of perceived exertion (sRPE) was utilised.

3.7.3.1 Global Positioning System

External load was measured by a GPS (MinimaxX S4, Catapult Sports, Melbourne, Australia) sampled at a frequency of 10 Hz, shown to provide accurate assessment of total distance and high-intensity activity in team sports (Rampinini et al., 2015). The micro-technology units were secularly positioned between the scapulae of subjects in a custom-made tight-fitting vest to reduce movement artefact, players were familiar with wearing the devices as they are worn during all field-based practices and match-play. Players wore the same units throughout data collection and were switched on at least 15 minutes prior to exercise commencement to ensure full satellite signal (number of satellites 14.4 ± 0.5; horizontal dilution of precision: 0.81 ± 0.10). Metrics used for analysis were exported from manufacturer software (Catapult Sprint, Version 5.1.7, Catapult Sports, Melbourne, Australia), the external load variables used and the descriptions are located in Table 3.4. Details for specific arbitrary and individual speed thresholds are outlined within the relevant chapters based on squad averages and individualised measures.

Table 3.4 External Training Load Variables from Global Positioning System Technology

Total Distance (TD)	Total distance covered in training or match play
Acceleration/Deceleration Load (AD Load)	Acceleration and deceleration distance ± 2 $m \cdot s^{-1}$
High Speed Running (HSR)	Distance covered or time spent above ~17 $km \cdot h^{-1}$
Very High Speed Running (VHSR)	Distance covered or time spent ~24 $km \cdot h^{-1}$
Distance Above MAS (m>MAS)	Distance covered above MAS $km \cdot h^{-1}$
Time Above MAS (t>MAS)	Time spent above MAS $km \cdot h^{-1}$

MAS, Maximal Aerobic Speed

External load variables derived from GPS data were input into outlined metabolic power equations (Osgnach et al., 2010) to calculate the internal metabolic cost of the activity ($kcal/kg^{-1}$). This method allows for the estimation of EE required to meet the training or match demands with the validity and variability previously investigated within football (Hoppe et al., 2017; Osgnach et al., 2010; Walker et al., 2016). In turn this estimation could be combined with the estimated RMR to estimate daily EE and in turn any ED outlined.

3.7.3.2 Session Rating of Perceived Exertion

Where GPS devices were not available players reported session rating of perceived exertion (sRPE) approximately 30 minutes after exercise completion and were verbally asked "how hard did you find the session?" in private by the principal researcher (Foster et al., 2001). Participants reported their overall session RPE using the modified CR-10 scale (Table 3.5), adapted from the Borg 10-point category-ratio scale, all participants were familiar with the use of RPE and this specific scale prior to study commencement due to frequent use within the training environment. Subsequently, RPE was multiplied by minutes played to calculate sRPE.

Table 3.5 Modified CR-10 Scale for Rate of Perceived Exertion (Foster et al., 2001)

Rating	Descriptor
0	Rest
1	Very, very easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	
7	Very Hard
8	
9	
10	Maximal

Chapter 4:
Evaluation of Blood Pressure and Axillary Body Temperature Assessment Techniques in Healthy Adults: A Method-Agreement Study

4.1 Introduction

Current assessment methods of thyroid function are not directly applicable to field-based testing of athletes; however, LC-MS analysis of THs, as well as BT and BP assessment could be viable alternatives. Current assessment of THs relies on venous blood sampling for analysis by ELICA and ELISA techniques to quantify circulating TSH levels and therefore have inherent limitations. Although efforts are being made to utilise more advanced analysis approaches, such as LC-MS, to determine T_4, T_3, rT_3 and T_2 concentrations, the reproducibility of this approach remains unknown and will be investigated in Chapter 5. One of the key advantages of LC-MS analysis is that the small blood volume required (~20 μL plasma) allows the use of finger-tip capillary samples which are both less invasive and simpler than antecubital-venous sampling (Nunes et al., 2006; Rondeel et al., 2010). Despite the relative efficiency of LC-MS analysis, it remains mildly invasive and requires the use of specialised equipment. Therefore, it could be beneficial to identify affordable, non-invasive surrogates for thyroid function and metabolic rate that particular individuals could utilise to monitor thyroid function when blood sampling is not feasible. This would be of particular relevance for populations in which thyroid function and metabolic rate could be at risk, such as adolescent athletes, where frequent TH analysis would be beneficial but not feasible.

Due to the role of the thyroid in energy production, it therefore mediates thermogenesis, thus many clinical manifestations of thyroid dysfunction appear as alterations in thermogenesis (Chatzitomaris et al., 2017; Landsberg, 2012; Silva, 2003; Simonsick et al., 2016). Accordingly, BT has been suggested to be indicative of metabolic rate and thyroid function (Chatzitomaris et al., 2017; Kim, 2008; Landsberg, 2012; Silva, 2003; Simonsick et al., 2016) and has previously been utilised in exercise studies (Soare et al., 2011). The agreement between BT and directly assessed TH concentrations, however, has received little attention. Approaches to BT assessment vary, with rectal temperature assessment by mercury-in-glass thermometers often regarded as gold-standard (Lodha et al., 2000). However, this does come with ethical, safety and cost concerns. Measuring axillary temperature (AT) is less invasive, more convenient than other sites of BT measurement and correlates highly with core BT (Giuffre et al., 1990; Lodha et al., 2000). Although AT agrees with rectal and core BT in infants and children (Lodha et al., 2000) when using mercury-in-glass thermometers, health and safety concerns about mercury

and glass thermometers has led to digital thermometers becoming more popular. Digital thermometers avoid the health and safety concerns of traditional mercury models and allow rapid BT assessment. However, validity is often unknown and can vary widely between devices (Craig et al., 2000). Thus, prior to the use of digital thermometers to assess AT as an indicator of thyroid function, it is vital to assess the agreement of these against gold-standard mercury-in-glass thermometers (Accoson, UK).

Further to the relationship between BT, EE and thyroid function, there is a reported association between BP and thyroid function, with evidence of a positive linear relationship between TSH and both SBP and DBP. Initial research in this area had inconsistent results with hypothyroidism (diagnosed with high circulating TSH), finding both supressed (Endo et al., 1979) and raised BP (Fommei & Iervasi, 2002; Saito et al., 1983). In contrast, it has also been noted that both clinical hyperthyroidism and hypothyroidism can present with elevated BP and therefore a greater prevalence of hypertension (Asvold et al., 2007; Fommei & Iervasi, 2002; Ittermann et al., 2012). Within normal reference ranges it has been reported that TSH has a positive linear relationship with both SBP and DBP, implying that subclinical hypothyroidism is associated with elevated BP (Asvold et al., 2007; Brock et al., 2012; Chen et al., 2012; Ittermann et al., 2012) specifically DBP (Fommei & Iervasi, 2002; Luke et al., 2004; Saito et al., 1983). Research in adolescents has not only replicated these findings, but also indicated that this relationship is stronger in males than females (Asvold et al., 2007; Chen et al., 2012; Ittermann et al., 2012). Therefore, the continued assessment of an individual's BP, specifically in adolescent males, could be used in conjunction with BT to monitor metabolic rate (Luke et al., 2004) and in turn estimate thyroid function (Ittermann et al., 2012) non-invasively. It can be hypothesised that acute increases in BP (specifically DBP) in conjunction with reduced BT could indicate decreased thyroid function and circulating TH. However, despite the ease of use with automated wrist-based sphygmomanometers, specific models have yet to be validated.

It is hoped that, should these tools be found to be valid measures of thyroid function, they could be implemented to monitor thyroid function in populations which could be at risk, specifically in adolescent athletes. Adolescent athletes are at risk due to the plethora of stress factors that occur for adolescent athletes including maturation, psychological stress, TL and inadequate EI, often resulting in RED-S, which can all supress thyroid function

and have subsequent implications for health and athletic performance (Briggs et al., 2015a; Elliott-Sale et al., 2018; Hackney & Dobridge, 2009; Hackney et al., 2012; Heikura et al., 2017; Kanaka-Gantenbein, 2005; Rubenstein & Butler, 1973; Taylor et al., 2017). In turn, assessment of thyroid function within adolescent athletes will be beneficial. Furthermore, continual assessment of thyroid function would allow for detection of periods where thyroid function could be supressed to a greater extent, potentially circa PHV, during congested fixtures or when under a greater ED.

The aims of this study were twofold and were: 1. To examine the agreement between digital (Omron Corporation, Tokyo, Japan) and mercury (Oral Association Medical M68525, New York) thermometers in the assessment of AT in a resting state and; 2. To assess the agreement between digital wrist-based BP monitors operating on oscillometric principles (Firhealth, China) and manual mercury (Accoson, UK) measures of BP in a similar resting state.

4.2 Materials and Methods

4.2.1 Participants

With institutional ethics approval, 20 healthy adults (eight males and 12 females) from the staff and students at Northumbria University participated. Participant details are provided in Table 4.1. Participants were excluded if they had any known thyroid dysfunction or cardiovascular problems, and all participants were informed of the purpose, procedures and risks before providing written informed consent.

Table 4.1 Participant Characteristics

	Method Agreement n=20
	Mean ± S.D
Age (y)	32 ± 12
Mass (kg)	75.2 ± 11.3
Stature (m)	1.77 ± 0.08
BMI (kg/m^2)	24 ± 3

BMI, body mass index

4.2.2 Study Design

Participants attended a single session at either their place of work or study where simultaneous measurements of AT were recorded using a digital (Omron Corporation, Tokyo, Japan) and mercury-in-glass thermometer (Oral Association Medical M68525, New York) in the same location, described below. Following this, BP was taken using a manual-mercury sphygmomanometer and stethoscope (Accoson, UK) and immediately afterwards (approx. 30 seconds delay) with a wrist-based sphygmomanometer (Firhealth, China).

Prior to measurements being taken, participants were sat upright with their arms resting on a surface level with their heart. Participants rested for 10 minutes and were encouraged not to talk during this time. Following this period of 10 minutes the mercury-in-glass thermometer was placed in the left axilla for a period of 10 minutes, described below. During this time multiple measures of BP were taken by both manual and digital methods and digital measurement of BT was taken. All measurements on each piece of equipment were taken three times with a mean of the measurements taken as the individuals score. See Figure 4.1 for a schematic of study design.

4.2.3 Body Temperature

After 10 minutes of seated rest in a room at ambient temperature, the mercury-in-glass thermometer (Oral Association Medical M684525, New York) was placed and held under the left axilla for 10 minutes, in line with manufactures instructions. In the final two minutes, an additional digital thermometer (Omron Corporation, Tokyo, Japan) was

placed under the left axilla, ensuring both thermometers tips were within close proximity. After the 10 minutes had passed, readings from both thermometers were recorded.

4.2.4 Blood Pressure

Alongside BT assessment BP was assessed using a digital wrist-based sphygmomanometer (Firhealth, China) and manual mercury sphygmomanometer and stethoscope (Accoson, UK) in rapid succession. Due to the nature of BP and the occlusion involved it is not feasible to take two simultaneous measurements, therefore only after the full release of one cuff could the other measurement be commenced. This in turn prevented venous congestion. The digital wrist-based sphygmomanometer was placed on the participants left wrist which was resting on a horizontal surface level with their heart. Subsequently, a BP cuff was positioned on the participant's upper right arm with the lower edge 1 inch above the antecubital fossa and a stethoscope placed on the brachial artery. BP was recorded by a trained researcher using a mercury sphygmomanometer (Accoson, UK) using the first and fourth Korotkoff sounds from the stethoscope to identify SBP and DBP respectively. On full release of the BP cuff, the digital wrist-based sphygmomanometer was switched on with the measurement recorded when displayed on screen in line with manufacturer's instructions. Participants rested for 2 minutes before measurements were repeated.

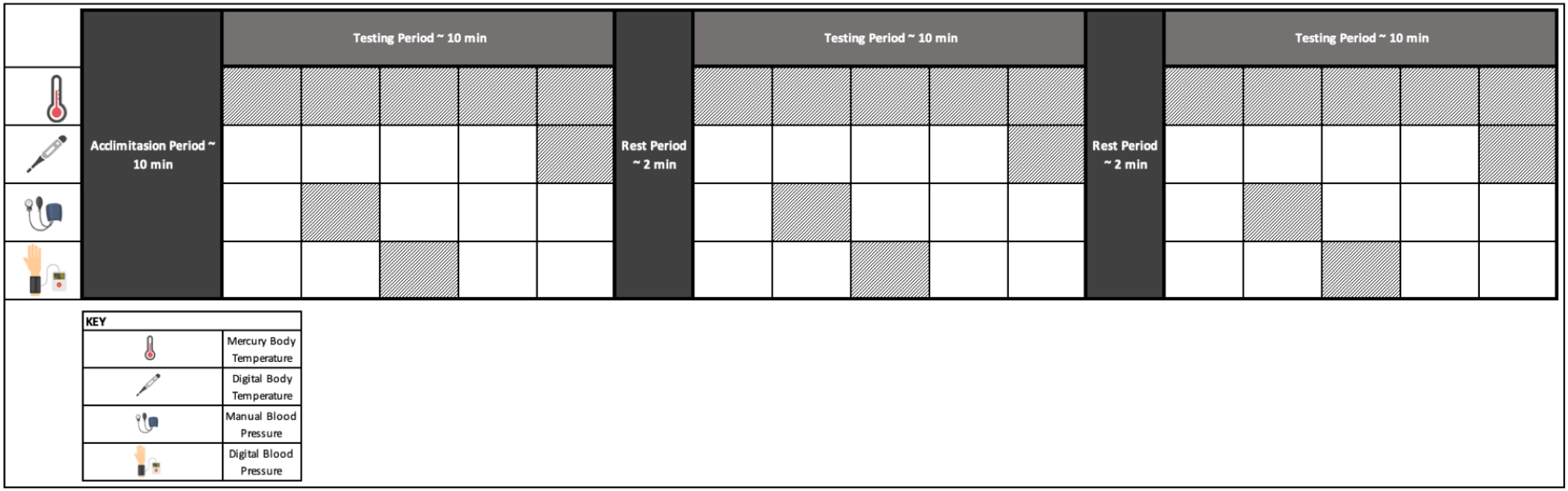

Figure 4.1 Schematic of Study Design.

4.2.5 Statistical Analysis

Statistical analysis was conducted using SPSS 24.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 8.0 (GraphPad Software, LA Jolla California USA). Data were presented as mean ± S.D. As power calculations are only necessary when testing a null hypothesis and when Type I and II errors are specified and controlled, which is not relevant within validity testing, sample size was estimated following recommendations (Hopkins, 2000). Concurrent validity between digital and manual measures of BT and BP were assessed using Bland-Altman limits of agreement (LOA) (Bland & Altman, 1986), typical error expressed as a coefficient of variation (CV, %) to quantify random error, and linear regression. The latter generated calibration equations to allow manual measurement scores (criterion measurement tool) to be predicted from digital measures. Systematic and proportional bias was assessed and visually presented by Deming regression (Deming, 1943).

4.3 Results

In total 20 participants completed the agreement study for both AT and BP assessment methods with agreement for BT, SBP and DBP described below.

4.3.1 Agreement between Mercury and Digital Measures of Body Temperature

Agreement between mercury (36.29 ± 0.24 ^{o}C) and digital (36.9 ± 0.2 ^{o}C) measures of BT were strong (CV_a = 0.3%, LOA ± 0.64 ^{o}C) - Figure 4.2, panel A. Deming regression analysis also revealed no evidence of systematic [intercept (95% CI) = -12.95 (-35.58 to 9.69) ^{o}C] or proportional bias [slope (95% CI) = 1.33 (00.72 to 1.95) ^{o}C] between mercury and digital measures of BT (Figure 4.2, panel B).

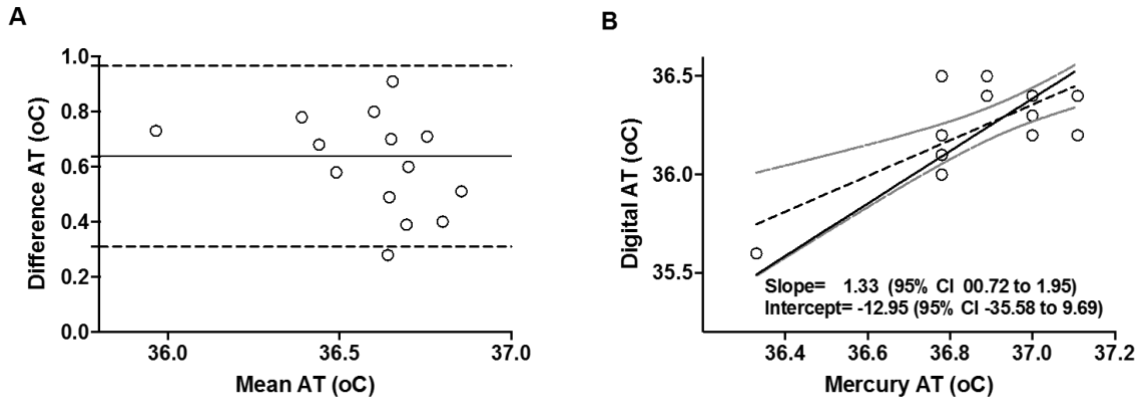

Figure 4.2 Comparison of Axillary Body Temperature (BT) (oC) Assessed by Mercury-in-glass and Digital Thermometers. (AT, Axillary Body Temperature)
A) Bland Altman Limits of Agreement – solid line indicating the mean and dotted lines denoting the upper and lower limits of agreement.
B) Demining Regression Analysis – solid line represents the line of equality, dashed blackline and corresponding grey dashed lines denote regression line with 95% confidence intervals

4.3.2 Agreement between Manual and Digital Measures of Blood Pressure

For SBP, agreement between mercury (122.2 ± 18.2 mmHg) and digital (115.3 ± 21.0 mmHg) measures were strong to modest (CV_a = 9.7%, LOA ± 6.90 mmHg), Figure 4.3, panel A. Deming regression analysis revealed no systematic (-32.40 (-97.71 to 32.91) mmHg) or proportional bias (1.21 (0.68 to 1.74) mmHg) between mercury and digital measures, Figure 4.3, panel B.

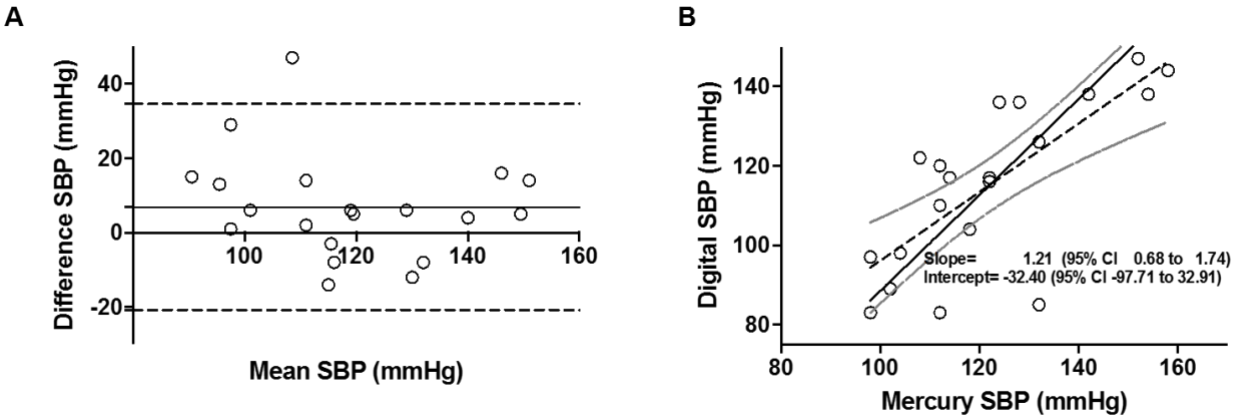

Figure 4.3 Comparison of Systolic Blood Pressure (SBP) (mmHg) Assessed by Manual Mercury Sphygmomanometer and Digital Wrist-based Sphygmomanometer.
A)Bland Altman Limits of Agreement – solid line indicating the mean and dotted lines denoting the upper and lower limits of agreement.
B) Demining Regression Analysis – solid line represents the line of equality, dashed blackline and corresponding grey dashed lines denote regression line with 95% confidence intervals

For DBP, agreement between mercury (76.7 ± 6.75 mmHg) and digital (74.1 ± 11.8 mmHg) measures were strong to modest (CV_a = 9.9%, LOA ± 2.60 mmHg), Figure 4.4, panel A. Deming regression analysis revealed no systematic (-100.60 (-205.00 to 3.74) mmHg) or proportional bias (2.28 (0.92 to 3.63) mmHg) between mercury and digital measures, Figure 4.4, panel B.

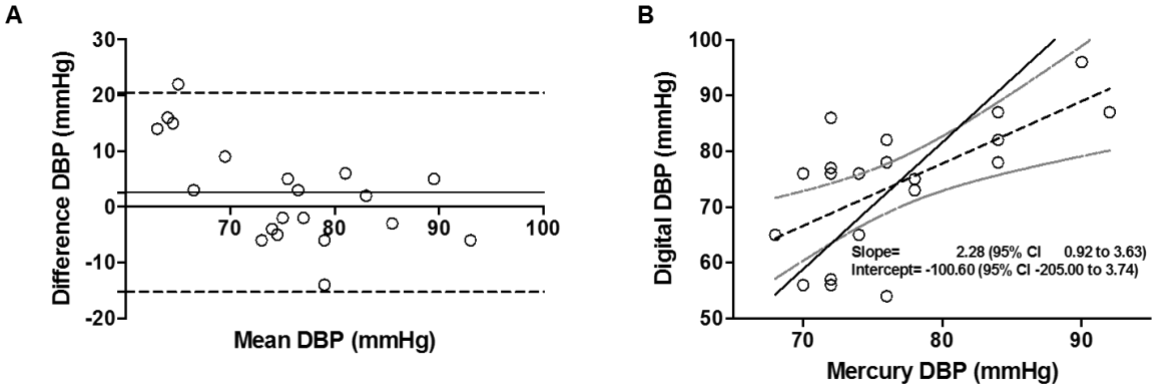

Figure 4.4 Comparison of Diastolic Blood Pressure (DBP) (mmHg) Assessed by Manual Mercury Sphygmomanometer and Digital Wrist-based Sphygmomanometer.
A) Bland Altman Limits of Agreement – solid line indicating the mean and dotted lines denoting the upper and lower limits of agreement.
B) Demining regression analysis – solid line represents the line of equality, dashed blackline and corresponding grey dashed lines denote regression line with 95% confidence intervals.

4.3.3 Calibration Equations

Calibration equations were generated using the findings from regression analysis to allow manual measurement scores (criterion measurement tool) to be predicted from digital measures. These are shown below and are used and referred to in future research as a predictor of actual BT and BP.

BT: *y = 1.33x – 12.95*

SBP*: y = 1.21x – 32.40*

DBP*: y = 2.28x – 100.60*

y = criterion measurement tool
x = digital measures

4.4 Discussion

This study examined the agreement between digital measures of BT and BP and gold-standard-manual measures of the same variables. The findings support the use of the digital tools for measurement of AT (Omron Corporation, Tokyo, Japan), SBP and DBP (Firhealth, UK) in healthy individuals when correction equations are used. These findings have practical implications for both researchers and practitioners who wish to quantify and monitor BP and BT in a feasible and affordable manor. In this study there was no systematic or proportional bias between measurement tools suggesting that, under standardised conditions, digital and manual measures are comparable.

4.4.1 Body Temperature

Typical error between AT measurements reported within these findings (0.12 ^{o}C) were lower than those outlined in similar validity studies utilising radiofrequency telemetered thermometers during indoor exercise (0.2 and 0.27 ^{o}C) (Ganio et al., 2009; Soare et al., 2011), indicating that digital assessment of BT by the Omron device (Omron Corporation, Tokyo, Japan) is valid for BT assessment. Results suggest that the digital thermometers utilised in this study (Omron Corporation, Tokyo, Japan) give valid results in comparison to mercury-in-glass thermometers when measurement is taken at the axilla. This study reports lower variance than previous method agreement studies (Ganio et al., 2009), however digital methods did record systematically lower BT than mercury-in-glass methods, similar to earlier core BT research (Giuffre et al., 1990). This can be accounted for through the use of correction equations due to the lack of systematic and proportional bias ($y=1.33x-12.95$, where y=mercury prediction of BT and x=BT by digital thermometer) to infer core BT.

Site of BT assessment is known to result in further fluctuations in both paediatric (Craig et al., 2000; Lodha et al., 2000) and adult populations (Giuffre et al., 1990). Core BT assessed by mercury-in-glass thermometers have reportedly assessed BT 0.19 ^{o}C higher than AT with the same thermometer, and when measured by digital thermometers AT was 0.33 ^{o}C lower that core by mercury-in-glass (Giuffre et al., 1990). The findings from this study support this earlier research that digital methods of AT consistently measure lower than mercury-in-glass.

Despite these findings indicating a high level of agreement between digital (Omron Corporation, Tokyo, Japan) and mercury-in-glass thermometers in the assessment of AT, prior to this method being utilised in longitudinally monitoring of AT, the reproducibility of the devices must be established. This is of particular importance due to the known circadian fluctuation in core BT (Clark & Kruse, 1990), therefore, Chapter 5 evaluates the reproducibility of the Omron thermometer in the assessment of AT within and between days.

4.4.2 Blood Pressure

The typical error of SBP and DBP in this study (9.99 and 6.44 mmHg respectively), and the tendency for the digital wrist-based BP monitors used within this study (Firhealth, UK) to over-predict both SBP and DBP reflects values reported within other method agreement studies. Similar changes in mean between digital and mercury BP devices were reported in a mixed cohort of 200 individuals (8.54 ± 9.38 and 4.21 ± 7.88 mmHg, for SBP and DBP respectively) and in parallel to this study, digital devices overestimated both SBP and DBP (Mansoor et al., 2016). Similarly, in hypertensive patients both SBP and DBP were over predicted by digital devices in comparison to mercury

sphygmomanometer, with mean±SD differences of 2.50 ± 5.30 and 1.40 ± 4.10 mmHg for SBP and DBP respectively (Altunkan et al., 2007). However, despite multiple studies indicating that digital devices, irrespective of the manufacturer and model, over predicting both SBP and DBP when compared to mercury devices this is not consistent within the literature. A study in healthy non-hypertensive adults reported a similar over prediction of SBP by digital devices but an under-estimation of DBP (Lim et al., 2015) when compared to mercury sphygmomanometers, with a discrepancy of 0-27 mmHg. Furthermore, in adolescents, the results for DBP appear to be reversed with digital devices predicting DBP higher than when measured by mercury sphygmomanometers, whilst SBP estimation is accurate reflected in typical error values of 0.30 and 6.90 mmHg for SBP and DBP respectively (Menezes et al., 2010).

Some of these findings could infer that hypertensive status impacts on this variation and accuracy of BP devices, with underestimation by digital devices occurring with greater BP (Braam & Thien, 2005). However, when cohorts are sub-divided into groups based on this (SBP ≤120 , 120-150, >150 mmHg) the discrepancy remained similar with a consistent trend towards over-reporting by the digital devices (Mansoor et al., 2016). Therefore, it seems that different makes and models of digital-automated BP devices have varying discrepancy with changes in mean between digital devices and mercury sphygmomanometers from -0.3 to 8.54 and -1.4 to 6.9 mmHg for SBP and DBP respectively. Therefore method agreement/criterion validity should be assessed in individual devices being used.

Although the findings from this study reflect results seen in earlier research and similar error values, they do not meet national standards for clinical validity in BP measurement.

The AAMI guidelines (White et al., 1993) state that both SBP and DBP should be within 5 mmHg with a S.D of 8 mmHg. In addition, the use of validation bands based on absolute values for SBP and DBP utilised by the European Society of Hypertension (ESH) and International Protocol for validation of BP monitors (O'brien et al., 2002) would classify the Firhealth wrist-based BP monitor as 'moderately inaccurate' due to the large S.D reported. However, as the implications of this study are not to interchangeably use the digital methods with mercury measures this does not have inherent limitations for future research. This is further supported by the low bias reported within this study (1.21 and 2.28 mmHg for SBP and DBP respectively) the correction equations reported above allow for correction of the systematic discrepancies shown from the manual-criterion measure, reducing incorrect diagnosis of hyper- or hypotensive status. Furthermore, in the wider context of this thesis, the digital method will monitor an individual's BP. If the measure is reliable (Chapter 5), it remains a logistical, affordable and feasible measure of BP monitoring.

4.4.3 Implications

Despite the FirHealth U60A wrist-based BP monitors having larger discrepancies when compared to manual methods in BP assessment they had low bias, consistently over predicted SBP and DBP in individuals. Importantly, due to the lack of systematic and proportional bias within the results and error values similar to those reported in previous findings, despite the device not being valid for clinical assessment of BP it still remains a possible feasible tool to be used in the monitoring of BP in individuals if results are found to be reproducible. In contrast the digital thermometers consistently under-predicated axillary BT when compared with mercury methods but similarly had low bias.

As such the following correction equations could be utilised to give a more accurate reflection of BP and BT:

BT: ***y = 1.33x – 12.95***

SBP***: y = 1.21x – 32.40***

DBP***: y = 2.28x – 100.60***

y = criterion measurement tool
x = digital measures

In the field and exercise setting, the availability of resources and time available is limited and therefore the ability to reliably measure BP and BT in under two minutes advances current practice in thyroid function assessment. Furthermore, the tools utilised within this study are practical alternatives for self-monitoring which can also be considered when implementing into practice if these methods are found to be reproducible between and within-days. Therefore, prior to using these methods in research studies and implementing them into athlete monitoring, it is vital to assess their reproducibility within and between days. This is evaluated in Chapter 5. As such, these devices are utilised in subsequent studies with the above calibration equations considered when interpreting and reporting findings.

4.5 Conclusion

In conclusion, the Omron digital thermometers have strong to modest validity when measurements are taken at the axilla in a healthy adult population, when compared to the gold standard mercury-in-glass thermometers. Findings from this study reported smaller variation than those previously reported however a consistent trend of under-prediction by the digital thermometers was noted. Therefore, the correction equation could be used to estimate true axillary temperature. Importantly, due to the lack of systematic or

proportional bias for the digital BP monitors and thermometers they could be utilised to monitor BT and BP in individuals if future research deems it a reliable assessment tool.

Chapter 5:
Within and Between-Day Reproducibility of Axillary Temperature, Blood Pressure Using Automated Devices and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS) to Assess Circulating Thyroid Hormone Concentrations in Adolescent Males

5.1 Introduction

In Chapter 4 of this thesis alternative methods of assessing BT and BP were examined in healthy adults, providing valuable insight into feasible methods of BT and BP monitoring that could be utilised by practitioners and researchers in field-based research. Results suggested that the use of digital thermometers to assess BT could offer an alternative to mercury-in-glass thermometers, despite the under-prediction of the digital thermometers. However, the reproducibility of the digital device needs to be examined. The Firhealth wrist-based BP monitor, while not satisfying AAMI and EHS guidelines for validity in BP measurement, displayed no proportional or systematic bias. However, over-prediction by the digital method was apparent in both SBP and DBP. Despite the lack of validity with BP monitors against published criteria, if over predictions are found to be consistent under controlled conditions and devices are found to be reproducible within and between days they could still be utilised as a feasible tool of monitoring BP in individuals with an appropriate calibration adjustment/offset.

Due to the logistical challenges of venous blood sampling, it is hoped that BT and BP assessments could be utilised as surrogates for monitoring thyroid function in adolescent footballers, therefore, it is important to assess the relationship between BT, BP and TH concentrations. Through assessing the reproducibility of AT and BP within and between days it is also possible to quantify natural biological variation in AT and BP, enabling the establishment of smallest meaningful/detectable change with this measure for future monitoring and intervention studies should it provide a useful surrogate for TH in adolescents as it is in adults (Soare et al., 2011). Thus, it is hoped that the continued assessment of an individual's BP, specifically in adolescent males, could be used in conjunction with BT to monitor metabolic rate (Luke et al., 2004) and in turn estimate

thyroid function (Ittermann et al., 2012). Ultimately implying that the combined monitoring of BP with BT could be affordable and non-invasive surrogates to monitor thyroid function when quantification of THs is not possible.

As discussed in section 2.5 there are multiple limitations to current methods of TH analysis. Therefore, the development of new techniques allowing the assessment of the whole suite of THs from a less invasive sampling method could allow for greater insight into thyroid function of those at risk of suppressed thyroid function, including adolescent athletes. Current assessment methods for thyroid function rely on ECLIA and ELISA methods and primarily determine circulating TSH and T_4 concentrations at one time point to diagnose thyroid dysfunction (Chatzitomaris et al., 2017; Thienpont et al., 2013b; Wang & Stapleton, 2010). However, it is becoming increasingly apparent that individuals can have inadequate thyroid function, and sub-optimal T_3 concentrations, despite having TSH and T_4 concentrations within reference ranges due to reduced peripheral conversion and therefore diagnostic tests relying on TSH and T_4 could be under-diagnosing hypo-metabolism (Abdalla & Bianco, 2014; Andersen et al., 2002; Jonklaas et al., 2014; Masika et al., 2016). Therefore, the measurement of T_3, rT_3 and T_2 in the diagnosis and monitoring of thyroid function is potentially more useful (Abdalla & Bianco, 2014; Li et al., 2014; Masika et al., 2016; Thienpont et al., 2013b; Wang & Stapleton, 2010).

In contrast to immunoassay analysis, LC-MS allows for the rapid assessment of T_4, T_3, rT_3 and T_2 from ~20 μl of plasma and has recently been validated in rats (Kunisue et al., 2010), sea lamprey (Bussy et al., 2017) and during pregnancy in humans (Kahric-Janicic et al., 2007) as well as in confirming the log-linear TSH and TH relationship in euthyroid patients (Jonklaas, 2008). A recent study from the laboratories utilised within the analysis

of our samples also found strong agreement between LC-MS methods and ELISA/ECLIA techniques in human serum ($r^2 > 0.99$ for all hormones) whilst also outlining the increased sensitivity and significant reduction in analysis time (Bowerbank et al., 2019). Furthermore, due to the poor sensitivity of immunoassays, they are not adequately reliable to determine FT_3 concentrations (Li et al., 2014; Masika et al., 2016), in particular at high and low concentrations (Bowerbank et al., 2019; Jonklaas et al., 2014; Masika et al., 2016), which are key to the diagnosis of thyroid dysfunction. In contrast, LC-MS is known to be highly accurate and sensitive even at low hormone concentrations (Bowerbank et al., 2019; Masika et al., 2016) . Therefore, despite not being able to measure the more traditionally used method of thyroid function (TSH), it could be argued that reliable assessment of circulating T_3 concentrations is a more useful measure of thyroid function and an individual's metabolic state. Furthermore, in conjunction with the assessment of T_4, rT_3 and T_2 it could be possible to identify the origin of thyroid problems, postulating peripheral conversion of T_4. Additionally, due to the reduced quantity of blood required for analysis, finger-tip capillary samples can be used, often with improved preference to venous samples, and samples collected more frequently. This has clear implications within the applied sport setting in which individual reference ranges can be noted and monitored longitudinally to identify time points in which thyroid function might be at risk, for example during injury or congested fixtures. Thus, the use of LC-MS could be a reliable alternative to assessing circulating TH (T_4, T_3, rT_3, T_2) concentrations.

It is known that TH concentrations have large individual variation in relation to sex, age and exercise status (Andersen et al., 2002; Hackney & Dobridge, 2009; Kanaka-Gantenbein, 2005; Kapelari et al., 2008; Rubenstein & Butler, 1973; Sapin et al., 1998)

and therefore the importance of individual reference ranges has been discussed (Andersen et al., 2002; Thienpont et al., 2013b). However, establishing reference ranges is not feasible using immunoassay analysis techniques due to the volume of blood and time required for analysis. In contrast, with the ability to utilise finger-tip capillary sampling, and the relatively small time needed for analysis, LC-MS could provide individual reference ranges or outline smaller population reference ranges. This is of notable importance in adolescents, as the implications of liver inefficiency, resulting in impaired deionisation of T_4 is compounded by factors including, growth, maturation and additional stress factors including exercise and a significant ED (Briggs et al., 2015a). Therefore, adolescent athletic populations could be at increased risk of suffering hypo-metabolism. The development of an easy, reproducible method of assessing metabolic rate and thyroid function in this population would be beneficial. However, the notable circadian fluctuations in TH concentrations (Abdalla & Bianco, 2014; Chatzitomaris et al., 2017; Russell et al., 2008) are not accounted for if a single assessment point is used. As such, the development of methods to monitor thyroid function should include multiple measurements over time. Quantifying oscillations over time is vital to establish normal variation in specific populations and to establish the magnitude of smallest meaningful changes. This, in turn could inform diagnosis, as well as further research within specific population groups where TH concentrations are the primary measure.

The aims of this study are threefold and are to: 1. assess the within and between-day reproducibility of BP and BT in adolescent males, including a sub sample of academy-level football players, without clinically-diagnosed thyroid dysfunction (part one); 2. assess the within and between-day reproducibility of circulating TH concentrations using LC-MS (part two) and; 3. assess the pattern of change of BT, BP and circulating TH

concentrations in adolescent males without clinically-diagnosed thyroid dysfunction over 24 hours (part two).

5.2 Materials and Methods

5.2.1 Participants

In total 26 healthy adolescent males participated in part one (15 ± 1 y), including 14 category one academy football players, with a subsequent sub-sample of 12 healthy-adolescent males participating in part two (14 ± 1 y). Participant details for each study are provided in Table 5.1. Inclusion criteria included a healthy BMI, based on the biological age and sex-specific classification for the United Kingdom (Cole et al., 2000), and no known thyroid problems.

In all studies, participants were informed of the purpose, procedures and risks associated with participation before written informed parental consent and participant assent were given. All studies were approved by the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria.

Table 5.1 Participant Characteristics by Study

	Part One	Part Two
	Reproducibility BP & BT	Reproducibility of TH and Association with BP & BT
	n=26	n=12
	Mean ± S.D	Mean ± S.D
Age (y)	15 ± 1	14 ± 1
Mass (kg)	69.51 ± 27.13	55.46 ± 12.98
Stature (m)	1.62 ± 0.29	1.64 ± 0.13
BMI (kg/m^2)	23.10 ± 5.45	20.42 ± 3.56
Age from PHV (y)	0.98 ± 1.04	-0.48± 1.27

BMI, body mass index; PHV, peak height velocity; BP, blood pressure; BT, body temperature; TH, thyroid hormones

5.2.2 Study Design

5.2.2.1 Part One

In part one, participants were provided with digital, automated devices to self-monitor BT (Omron Corporation, Tokyo, Japan) and BP (Firhealth, China). These devices were previously validated (Chapter 4) against a mercury-in-glass thermometer (Oral Associations Medical M68525, New York) and a manual mercury sphygmomanometer (Accoson, UK). Participants were visually and verbally instructed on the correct use of the devices and when the measures should be taken. Both BT and BP were recorded at all three test points. Measures were taken at baseline (approximately three hours after waking), 80 minutes and 24 hours (t=0, t=80, t=1440 minutes), this allowed for the assessment of within and between-day reproducibility of self-monitoring digital BP and BT. A schematic of the study design is shown in Figure 5.1. Time points were chosen to reflect those that will be used in future studies in this thesis, dictated by the length of a standard football match in these age groups and allowing for 24 hour follow-up measurements after an exercise bout and subsequent stress exposure. Participants were

instructed to refrain from formal training or strenuous PA until all testing had been completed and were encouraged to maintain other habitual daily activities and dietary intake throughout the study. Participants were also instructed to maintain the same breakfast on both test days, with compliance verified by a food recall when equipment was returned approximately 24-72 hours after study completion. To reflect time points covered in study two, participants were encouraged that t= 0 min and t =1440 minutes were approximately three hours after waking.

5.2.2.2 Part Two

In part two, all data were collected in the participant secondary school that participants attend as normal, and three test points replicating study one were undertaken across 24 hours. At all three test points a ~500 ul fingertip capillary blood sample was taken by a qualified researcher, along with digital measures of BP (Firhealth, China) and axillary BT (Omron Corporation, Tokyo, Japan). Participants were instructed to attend school on the first day of testing after consuming their habitual breakfast and travelling to school in their usual manner. This was replicated on the second day of testing. Compliance was determined through a food and activity recall on both test days. Between t=0 and t=80 minutes, participants were instructed to attend formal lessons but refrain from consuming food or liquids other than water and not to undertake any irregular PA. School teachers were aware of the research criteria and assisted adherence. A schematic of study design is shown in Figure 5.2.

In the broader sense of this thesis, both studies would have ideally been undertaken in the controlled setting of the football academy assessing adolescent male footballers, which were used for subsequent studies. This would have allowed for individual reference

ranges to be outlined and true individual variation noted. However, due to the training demands on the players it was not possible for the players to be rested under the controlled conditions as necessary to assess this biological variation. Therefore, a school-based population, closely matched to the academy population for chronological and biological age, were used to assess the reproducibility of measures.

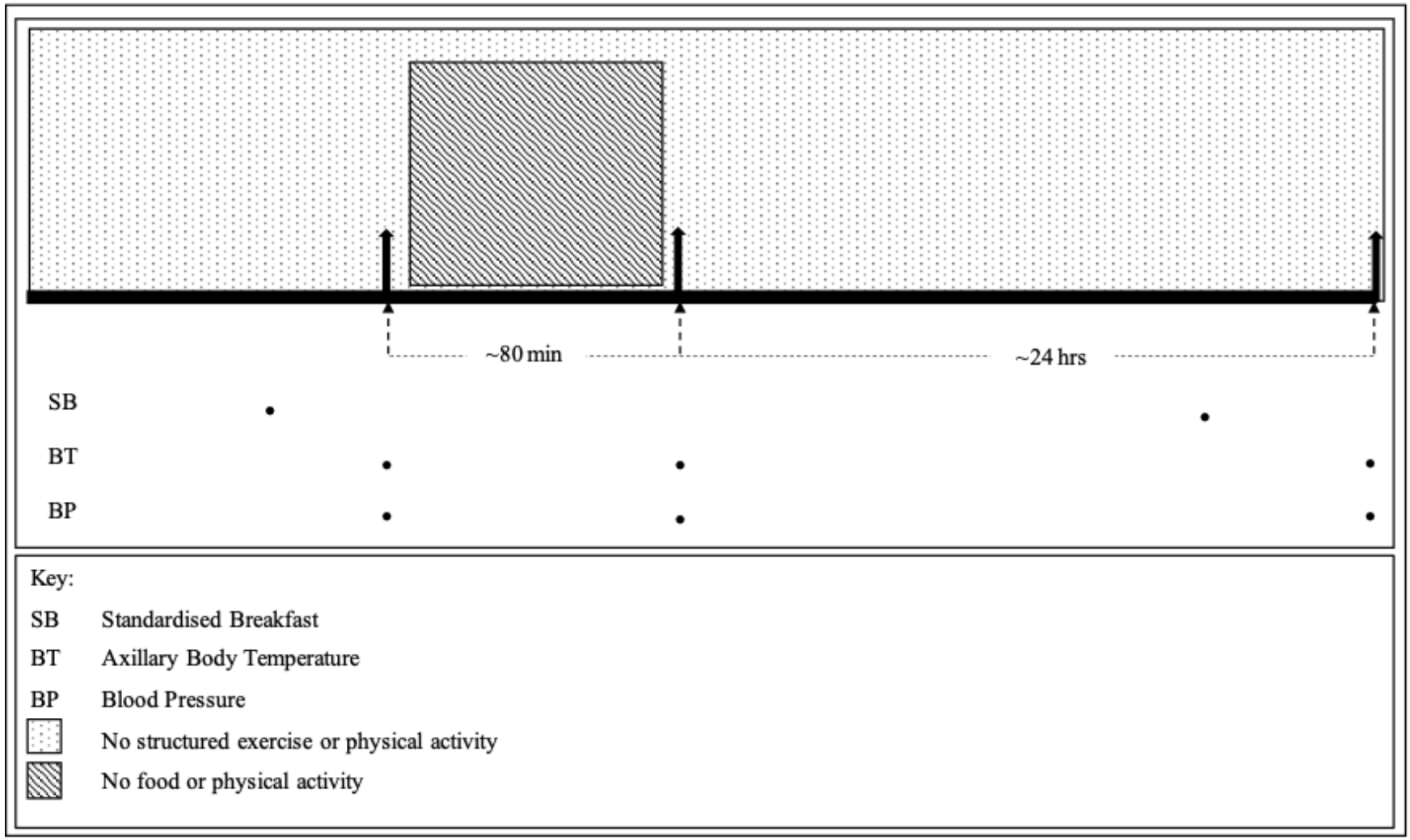

Figure 5.1 Schematic of Study Design for Part One.

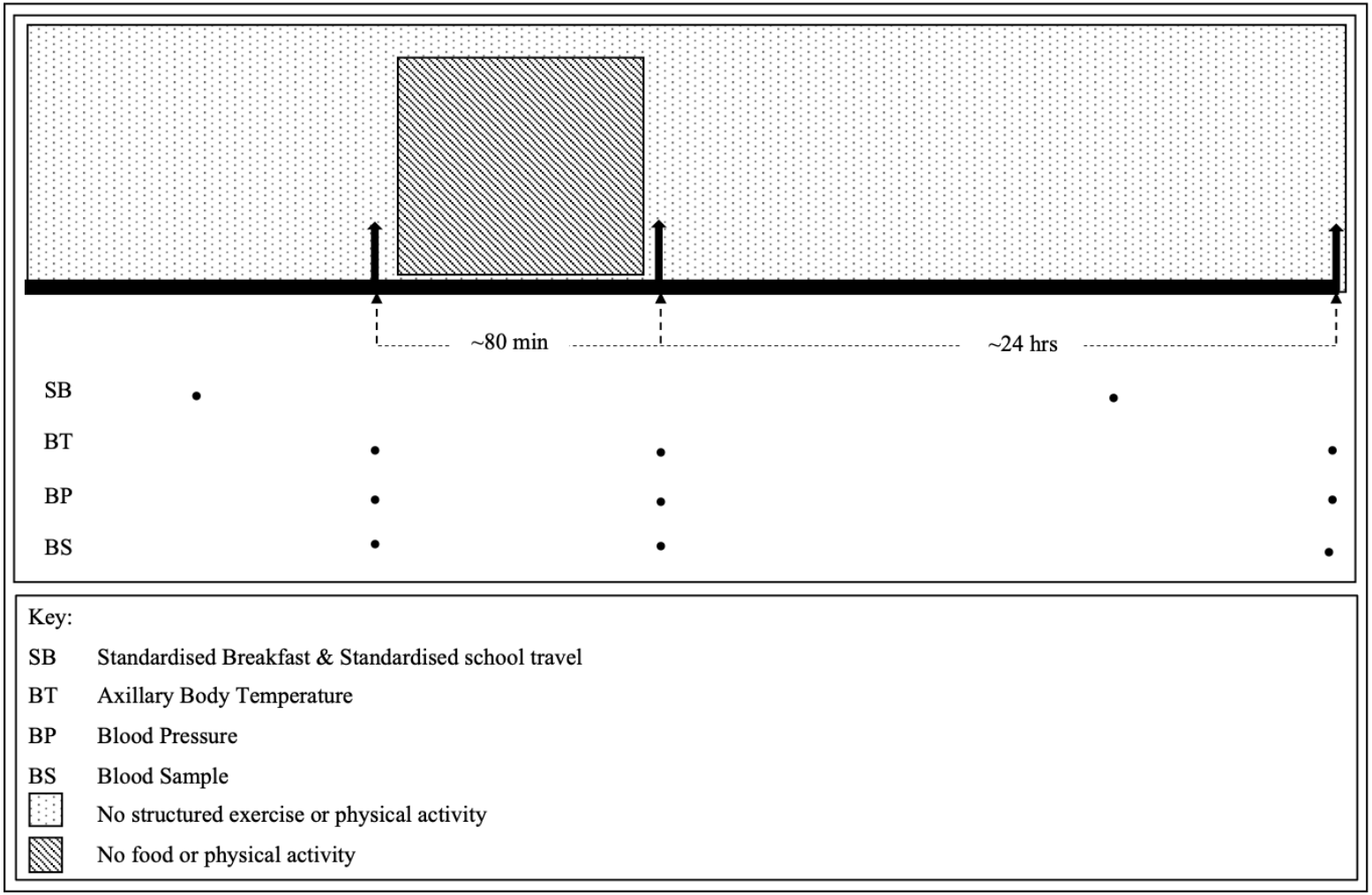

Figure 5.2 Schematic of Study Design for Part Two.

5.2.3 Biological Age

Anthropometric data were taken according to ISAK procedures. Standing and seated stature, measured to the nearest 0.01 m using a Harpenden Portable Stadiometer (Holtain Limited, Pembs, UK) and BM, measured to the nearest 0.1 kg using portable SECA scales (SECA, UK), were used to calculate BMI (kg/m^2) and age from PHV (y) as a measure of biological age (Mirwald et al., 2002).

5.2.4 Blood Pressure

In both parts, BP was measured using a digital wrist-based sphygmomanometer (Firhealth, China) as described in Section 3.4 at t=0, t=80 and t=1440 minutes. Due to logistical issues preventing participants to attend the academy during these windows participants were required to self-monitor and report their results. Participants were instructed verbally and visually on the correct methods for assessing their own BP, participants subsequently showed the researcher they were capable of doing so before taking the device away. In part one, participants were responsible for taking their own BP, whilst in part two participants were supervised at each time point by a researcher.

5.2.5 Axillary Body Temperature

Similar to the BP assessment in both parts, AT was assessed at t=0, t=80, t=1440 minutes. Participants were instructed verbally and visually on assessing their own AT using a digital thermometer (Omron Corporation, Tokyo, Japan) and indicated to the researcher they were capable of doing so correctly. In part one, participants were responsible for measuring their own AT whilst in part two, two participants were supervised at each time point by a researcher.

5.2.6 Blood Sampling

In part two, TH concentrations were assessed from ~500 µl of whole blood collected via fingertip-capillary sampling (providing ~200-300 µL of plasma for subsequent analysis), at each of the three test points as described in section 3.5.

5.2.7 Statistical Analysis

Statistical analysis was conducted using a custom-made spreadsheet (Microsoft Excel, Microsoft Corporation, Washington) and SPSS 24.0 (SPSS, Inc., Chicago, IL). Descriptive data were calculated as mean ± SD. As power calculations are only necessary when testing a null hypothesis and when Type I and II errors are specified and controlled, which is not relevant within reliability testing, sample size was estimated following recommendations (Hopkins, 2000). All variables were tested for normality using Shapiro-Wilk tests, SBP, DBP and BT variables were normally distributed and therefore between and within-day reproducibility of SBP, DBP and BT was assessed for systematic error by paired-samples t-tests (significance accepted $p < 0.05$) and for within-participant (random) error using typical error (TE) in absolute units of measurement and expressed as % coefficient of variation (CV). For interpretation of CV the following cut-points allowed for interpretation as low (<5%), modest (5-15%) and high (>15%) for random error (Atkinson & Nevill, 1998; Hopkins, 2000). In addition to providing greater contextual insight the following were considered when interpreting TE data, in line with specific variables: low (0-5 mmHg, 0-0.5 ^{o}C), modest (6-8 mmHg, 0.5-1 ^{o}C) and high (> 8 mmHg, >1 ^{o}C) for BP and BT respectively (Ganio et al., 2009; O'brien et al., 2002; White et al., 1993). In addition, although not a primary aim of this study the correlation of TH concentrations and proposed surrogate markers (BT, SBP and DBP) was assessed via Pearson correlation analysis of the change between time points. Correlation was interpreted as strong where $r > 0.5$, modest where $0.3 < r < .5$, small where $r < 0.3$ (Cohen,

1988), due to this not being the primary aim of this study only significant or strong correlations are reported.

Within part two, there were extreme outliers for rT_3 and T_2, identified as three deviations from the interquartile range (Tukey, 1977). These outliers were believed to be due to problems with blood sampling resulting in analysis errors <LOQ, therefore these data points were removed from analysis due to the inability to interpret them (Weisberg, 2005), as such sample size was restricting for rT_3 (n = 7) and T_2 (n = 10). Following elimination of outliers T_2 variables violation of normality assumptions remained, which was not rectified by log-transformation, but bootstrapping normalized the distribution of scores. Therefore, within and between-day reproducibility of FT_4, FT_3, rT_3 and T_2 were assessed for systematic error using bootstrapped paired-samples t-tests (significance accepted at $p > 0.05$) and for within-participant (random) error using typical error in absolute units of measurement and expressed as % coefficient of variation (CV).

5.3 Results

5.3.1 Part One

In total, 26 participants completed the reproducibility study for both BT and BP within and between days, including a subset of 14 category one, academy-level football players. Overall, reproducibility across 24 hours was modest for all measures. There was no significant change in BT, SBP or DBP over 80 minutes or 24 hours ($p \geq 0.05$ for all, range = 0.51-0.79). Within-participant random error of BT, SBP and DBP was modest to low (Table 5.2).

5.3.2 Part Two

In total a subsample of 12 participants from part one also completed the reproducibility study for thyroid hormone analysis by LC-MS. There was no significant change in any TH within days ($p \geq 0.05$ for all, range = 0.53-0.81) or between days ($p \geq 0.05$ for all, range = 0.57-0.90). FT_4 and FT_3 had the lowest typical error with higher random error in the measurement of rT_3 and T_2 (Table 5.3). The pattern of change in BT paralleled that of both FT_3 and FT_4 (Figure 5.3), with correlation analysis indicating a moderate negative correlation between changes in BT and FT_4 ($r = -0.50$) and FT_3 ($r = 0.37$). The pattern of change for SBP indicated a strong significant negative correlation for FT_4 ($r = -0.70$, $p = 0.12$) and a non-significant strong correlation for FT_3 ($r = -0.56$) (Figure 5.4). For DBP the correlation analysis indicated a strong negative correlation for FT_4 ($r = -0.533$) and FT_3 ($r = -0.66$) (Figure 5.5).

Table 5.2 Within-participant (random) Variation of Body Temperature and Blood Pressure Assessed by Digital Methods over 24 hours in Adolescent Males (n=26)

	T = 0 min		**T = 80 min**				**T = 1440 min**			
	Raw Mean ± SD	***Corrected Mean***	**Raw Mean ± SD**	**TE (90% conf. limits)**	**CV (%)**	***Corrected Mean***	**Mean ± SD**	**TE (90% conf. limits)**	**CV (%)**	***Corrected Mean***
BT (ºC)	34.86 ± 0.74	*33.41*	34.90 ± 0.90	0.54 (0.44-0.71)	1.13	*33.47*	34.00 ± 0.70	0.54 (0.44-0.71)	1.21	*32.27*
SBP (mmHg)	122.69 ± 15.55	*116.05*	119.60 ± 24.40	16.68 (13.59-21.82)	9.39	*112.32*	121.30 ± 22.00	18.31 (15.19-24.39)	9.34	*114.37*
DBP (mmHg)	71.96 ± 12.34	*63.47*	75.20 ±1 3.20	11.48 (9.35-15.01)	11.06	*60.86*	73.70 ± 13.00	10.38 (8.46-13.57)	11.32	*67.44*

***TE** and **CV (%)** values depict change from t =0 min.*

Corrected means based on correction equations outlined in Chapter Four
BT, body temperature; SBP, systolic blood pressure; DBP, diastolic blood pressure; TE, typical error; CV, coefficient of variation

Table 5.3 Within-participant (random) Variation of Thyroid Hormones (FT_4, FT_3, rT_3 and T_2) Assessed by LC-MS over 24 hours in Adolescent Males (n =12, 12, 7 and 0 for FT_4, FT_3, rT_3 and T_2 respectively).

	T= 0 min	T = 80 min			T = 1440 min		
	Mean ± SD	Mean ± SD	TE (90% conf. limits)	CV (%)	Mean ±SD	TE (90% conf. limits)	CV (%)
FT_4 (pmol/L)	21.55 ± 9.65	20.70 ± 12.10	6.00 (4.49-9.30)	28.67	20.30 ± 12.30	6.25 (4.68-9.70)	23.12
FT_3 (pmol/L)	5.53 ± 1.15	5.40 ± 1.70	1.58 (1.18-2.45)	24.74	5.60 ± 1.80	1.32 (0.98-2.04)	21.06
rT_3 (pmol/L)	38.71 ± 12.93	28.80 ± 17.20	13.75 (9.49-26.32)	33.81	30.10 ± 22.90	18.77 (12.95-35.94)	45.70
T_2 (pmol/L)	104.91 ± 11.47	91.30 ± 23.50	12.42 (9.06-20.44)	12.82	105.90 ± 30.10	21.96 (16.02-36.13)	15.91
T_4:T_3 ratio	4.07 ± 2.29	3.60 ± 1.30	1.34 (1.00-2.07)	23.66	3.50 ± 1.20	1.43 (1.07-2.21)	25.71
T_4:rT_3 ratio	2.00 ± 0.51	2.00 ± 0.80	0.43 (0.29-0.89)	30.31	1.90 ± 1.30	1.05 (0.71-2.20)	17.31

***TE** and **CV (%)** values depict changes from t = 0 min*

FT_4, Free Thyroxine; FT_3, Free Triiodothyronine; rT_3, Reverse Triiodothyronine; T_2, 3,5-Diiodothyronine; TE, typical error; CV, coefficient of variation

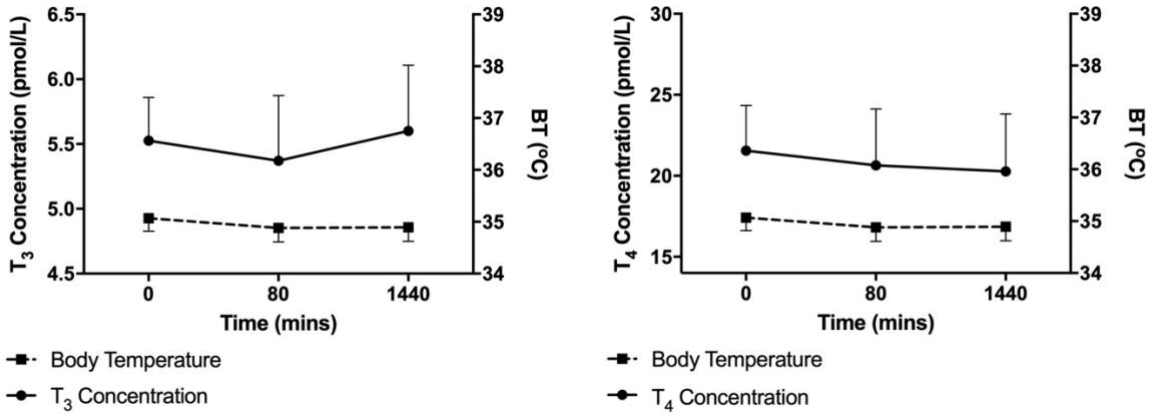

Figure 5.3 Change in Thyroid Hormones and Body Temperature (n=12).

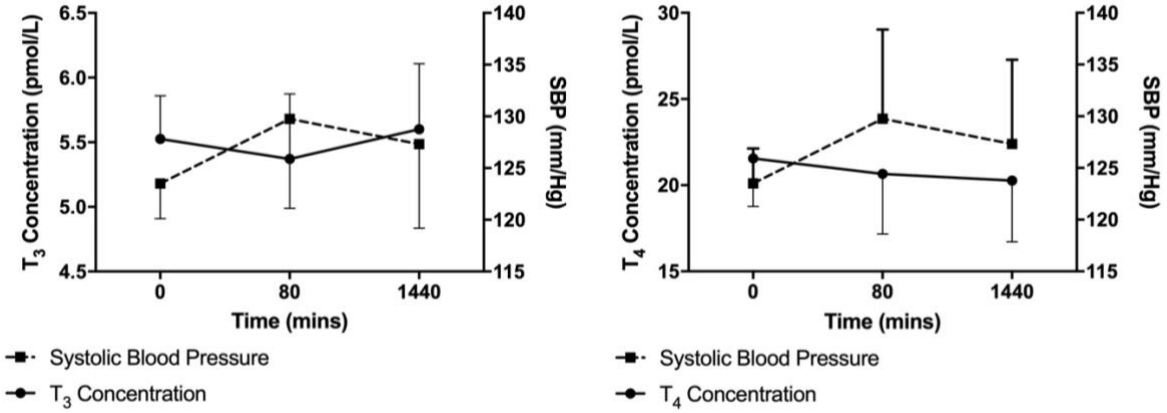

Figure 5.4 Change in Thyroid Hormones and Systolic Blood Pressure (n=12).

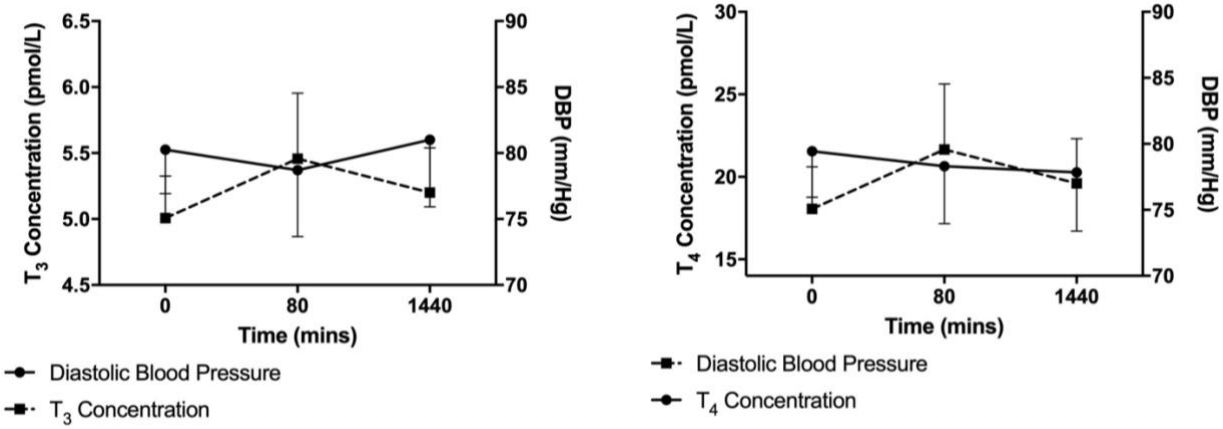

Figure 5.5 Change in Thyroid Hormones and Diastolic Blood Pressure (n=12).

5.4 Discussion

The aims of this study were to quantify within and between day reproducibility of circulating TH (FT_4, FT_3, rT_3, T_2) concentrations, using LC-MS, and BT and BP using automated-digital devices in adolescent males without clinically diagnosed thyroid dysfunction, and to examine the pattern of change of these variables relative to each other. This study aimed to identify potential surrogates and alternatives to current methods of assessment of thyroid function (ELICA and ELISA methods), to those that are more applicable to field research or sporting environments. The findings indicate that in healthy adolescent males, LC-MS analysis is a reproducible method of assessing circulating thyroid hormones, specifically FT_3 which, as previously outlined, is the primary determinant of metabolic rate. Moreover, the research supports the use of digital BP and BT as a surrogate method for continual monitoring of thyroid function in healthy-adolescent males, which could be utilised by young athletes and their coaches and support staff for identifying acute suppression of thyroid function.

There was no significant change in THs, however within-subject random variability was moderate to high depending on the hormone assessed. Arguably the TH with greatest biological importance is FT_3, which showed low to moderate random variation across 80-minutes and 24-hours, similarly FT_4 showed low to moderate random variation across the same time points and therefore these reproducibility metrics support the use of LC-MS in the analysis of FT_4 and FT_3. However, further metabolites of T_4, rT_3 and T_2, showed large random variation both within and between-day and therefore the use of LC-MS in the analysis of these two hormones must be interpreted with the large variation in the measures noted. This greater variation could in part be due to lower circulating concentrations of rT_3 and T_2 compared to FT_4 and FT_3. As our data demonstrate good reproducibility of FT_3 in finger-tip capillary samples analysed by the LC-MS method, our findings indicate that this method is valid for the assessment of key mediators of thyroid function and health whilst allowing for substantially smaller volumes of blood to be collected in a less-invasive manor.

Similarly, the digital thermometers and wrist-based sphygmomanometers showed no systematic bias within and between days, implying that these methods are reliable

between visits if conditions are standardised. Despite the multitude of environmental factors that can cause acute fluctuation in BT and BP, random error over a 24 hour period was very low for BT (CV=1.21%) and was only slightly higher for SBP (CV=9.34%) and DBP (CV=11.32%). Within adolescent male footballers, a consideration for future research in the broader sense of this thesis is the transient impact of exercise and nutrient intake on BP and BT. It could be expected that following a bout of PA, BP will increase acutely and, despite players subjectively feeling an increased temperature, their core BT might be suppressed in concurrence with suppressed FT_3. However, this particular study controlled for exercise with participants resting prior to samples being obtained and no structured or strenuous PA being undertaken on either day of testing. However, compliance to this was only confirmed qualitatively through asking the participants about their activities between testing points, quantitative assessments of PA were not taken within this research to limit participant burden.

Our findings support the link between FT_3, BT, SBP and DBP suggesting that within a healthy population BT and BP could offer useful surrogates for monitoring thyroid hormone fluctuations. Further research across clinically diagnosed hypo-, eu- and hyper-thyroid individuals would offer a valuable insight into whether BT and BP can predict diagnosed conditions, however this was not feasible within this study or thesis. The parallel and mirrored change in FT_3 and BT and FT_3 and SBP and DBP respectively were as expected from studies in adults (Asvold et al., 2007; Fommei & Iervasi, 2002; Luke et al., 2004; Saito et al., 1983) and children and adolescents (Chen et al., 2012; Ittermann et al., 2012) and support the use of BT and BP as surrogate markers of TH status in adolescent males.

Together, these results have implications for researchers wishing to analyse more than one TH in a less invasive, cost and time effective manner, and importantly for applied settings, where simplistic inexpensive methods can be used to monitor TH fluctuations in populations where it is beneficial to avoid taking blood samples for (e.g. pediatrics, athletes, adolescents and the elderly).

5.4.1 Body Temperature

Body temperature recorded at the axilla was the most consistent measurement in our population. Within-day there is a known circadian fluctuation in core BT (Sharma et al., 2015) that will in turn cause natural variation of axillary temperature (AT). This might account for the small within-day variation in our dataset, and due to the lack of systematic bias this value (0.54 ^{o}C), could be taken to be natural within-day variance of AT in this population.

The between-day variability cannot be attributed to this circadian variation and as such our results could suggest that 24 hour variation could solely be due to random error. Alternatively, it could be hypothesised that this variability is synonymous with changes in thermogenesis (Sharma et al., 2015). Notably, the variation in AT (change in mean = 0.08^{o}C) is paralleled by the within and between-day variation in T_3 **(**Figure 5.3a**)** supporting the suggestion that the minor variation in AT, and implied core BT, could be accounted for by changes in thermogenesis caused by simultaneous changes in T_3 concentrations (change in mean = 0.07 pmol/L). However, there was a large individual variation noted in FT_3 concentrations and AT changes across time points. Therefore, despite the overall tendency for T_3 and AT to be suppressed after 80 minutes of fasting and inactivity and remain consistent between days, individual responses must be noted. This must be considered in future research and highlights the importance of quantifying individual reference ranges for T_3 before examining the response to various stressors.

Despite an attempt to control for external factors known to mediate thyroid function and consequently thermogenesis, including EI and EE between measurements, minor variations remained, indicating biological variation. There is a lack of similar reproducibility studies outlining the acceptable biological variation of BT assessed by digital thermometers at the axilla, and the stated results indicate a consistent measurement in standardised conditions. The chosen measurement site of BT could account for some of the random error observed within this study as this is known to cause variations amongst BT assessment. Despite the ease, comfort and speed of digital axillary BT measurement (Lodha et al., 2000), unlike core BT it is influenced by air temperature, skin temperature, sweating and skin blood flow (Casa et al., 2007). These reasons could in part

be responsible for the reported under-prediction of core BT when assessed at the axilla (Casa et al., 2007; Ganio et al., 2009). Despite this, the reported methods remain a viable monitoring tool due to the consistency between and within-days considering ±0.54 ºC as the smallest meaningful change. Therefore, this is an acceptable approach to quantify BT at the axilla, however this should not be interchanged for measurement at different sites. Furthermore, correction equations such as those stated in Chapter 4, can be calculated for populations against mercury-in-glass thermometers at the same site to allow for a more accurate representation of gold standard axillary temperature

5.4.2 Blood Pressure

Similarly to digital methods of BT assessment at the axilla, automated wrist-based BP devices offer an appropriate method of self-monitoring BP in individuals. The findings stated above indicate that the Firhealth wrist-based sphygmomanometers can be used as a reliable measurement tool in the monitoring of BP in healthy adolescent males. However, the slightly larger typical error (18.31, 11.48 mmHg for SBP and DBP respectively) must be noted when interpreting changes between measurements. This can be expected due to the multitude of factors that can acutely impact on BP measurement that are often not possible to control. Although the random error for BP does have implications for future research, it must be noted that these methods can still be used to monitor BP longitudinally and to infer thyroid function if these typical error values are taken into consideration when interpreting changes over time.

In relation to using BP to assess thyroid function, both SBP and DBP inversely tracked T_3 (Figure 5.4, 5.5) and therefore support the use of BP monitoring as a surrogate marker of thyroid function. This finding supports more extensive studies identifying elevated BP with suppressed T_3 still within reference ranges (Asvold et al., 2007; Chen et al., 2012), subclinical hypothyroidism (Ittermann et al., 2012) and overt hypothyroidism (Fommei & Iervasi, 2002; Saito et al., 1983). Triiodothyronine (T_3) is known to have dilatory effects on vascular smooth muscle (Ojamaa et al., 1996) therefore reducing SBP and DBP. In turn, this apparent increase in SBP and DBP with decreased T_3 could be attributed to lower energy preventing the relaxation of smooth muscle in arterioles

resulting in a subsequent elevation in BP (Chen et al., 2012; Dagre et al., 2005; Danzi & Klein, 2003).

Noradrenaline, cortisol and aldosterone are also heightened in both hypothyroidism and under psychological stress (Fommei & Iervasi, 2002) with these steroid hormones known to increase BP (Lambiase et al., 2012). However, catecholamines were not measured in this study so this potential mechanism is speculative. However, this will be investigated in subsequent chapters of this thesis, not only providing an insight into the relationship between stress, cortisol and thyroid function but potentially greater understanding of the associations reported in this study. Despite this lack of mechanistic insight, the results of this study adds to existing research indicating a link between BP and thyroid function, specifically within adolescent males who have previously been reported to show a greater correlation between BP and TH than females (Chen et al., 2012). In turn, this research supports the use of BP monitoring as a logistically viable method of monitoring thyroid function, however, especially if measured in isolation, typical error values must be considered when interpreting results.

5.4.3 Thyroid Hormones

As examined in detail there are limitations in the current methods of diagnosing thyroid dysfunction and quantifying circulating TH concentrations, additionally, there are issues with current non-specific or individualised reference ranges. Therefore, it was hoped that LC-MS analysis would enable the examination of a more comprehensive suite of THs from a less invasive sampling method requiring a smaller quantity of blood. This study indicates LC-MS analysis can quanitfy FT_4, FT_3, rT_3 and T_2 from ~20 μL obtained from finger-tip sampling methods and that this sampling method has strong compliance in children and adolescence and is feasible within the field setting. The benefits of LC-MS in the analysis of TH concentrations are therefore apparent however the test-retest variability of the individual hormones in adolescent males must be considered when interpreting future findings. In addition, future research into group variances in TH concentrations might consider log transformation of data to account for the large individual variation reported within these findings.

5.4.3.1 Thyroxine and Triiodothyronine

There is an apparent need for individual reference ranges for TH concentrations (Andersen et al., 2002; Thienpont et al., 2013b), and studies attempting to identify narrower population reference ranges for the pediatric population (Kapelari et al., 2008; Lem et al., 2012) have acknowledged substantially smaller values for both FT_4 and FT_3, with similar ranges to those reported here.. Despite these smaller raw values, in this study the between-day reproducibility of FT_4 and FT_3 gave relatively small typical error values (6.25 pmol/L and 1.32 pmol/L respectively) indicating that LC-MS analysis constitutes a reproducible approach to FT_4 and FT_3 analysis in healthy adolescent males. However, there is still substantial variability in both baseline TH status and fluctuations in TH concentrations, both in direction of change and significance. Although the mean implies that there is a mirroring of BP and FT_3 concentrations, this is not always the case. This highlights the importance of noting an individual's regular FT_3 concentration and also how this responds to varying conditions, such as fasting, and noting their own natural fluctuations. Due to the noted impact of maturation on thyroid function it was of interest to investigate if stage of maturation had an impact on the natural biological between and within-day FT_3 variation. However, when accounting for maturation in relation to PHV (Mirwald et al., 2002) there was no apparent trend for pre, post or circa PHV. Therefore, although maturation is known to impact on thyroid function, this does not seem to influence the 24-hour fluctuation, and this is inherently an individual reaction.

The TE within days was larger than between day which could reflect the apparent circadian rhythm previously reported in circulating T_3 concentrations (Abdalla & Bianco, 2014). This in part could explain the parallel fluctuations in BT and T_3 (Figure 5.3a) and as explained, supports the notion that the circadian rhythm of BT is in part due to thermogenesis due to biological variation in thyroid function.

5.4.3.2 Reverse-triiodothyronine and Diiodothyronines

In addition to the benefits of the capillary finger-tip sampling technique, the use of LC-MS successfully allowed for the quantification of an array of TH. Although the reliable analysis of FT_3 is arguably the most important, due to it having the greatest impact on metabolic rate, the advantage of being able to distinguish T_4, rT_3 and T_2 values through

the same method adds depth to the data and provides greater mechanistic insight into why an individual might have suboptimal T_3 levels. For example, increased levels of rT_3, an inactive metabolite of T_4, can indicate if an individual has preference towards inner ring deiodination of T_4 by type 3 iodothyronine deiodinase enzyme. Thus, a greater amount of rT_3 is produced with a concurrent detrimental impact on T_3 levels, potentially in an effort to conserve energy during times of stress, disease or energy deprivation (Van den Beld et al., 2005). However, the results from this study do indicate a substantially higher CV (45.70%) for rT_3 than any of the other hormones. This could be, in part, due to the small sample size (n=7) or multiple factors that can have an acute impact on rT_3 concentrations. One possible reason for this could be the anticipation of blood sampling in the participants, inducing an acute bout of stress and therefore increasing rT_3. This is apparent at t=0 minutes and rT_3 decreases at each subsequent time point, potentially as participants become more familiar, and relaxed, with the sampling process, however this is speculative.

5.4.3.3 FT_4:FT_3 and FT_4 to rT_3 Ratio

The use of ratios within endocrinology allows a simple way to analyse the effects of two interdependent hormones and to further comprehend their dynamic relationship (Sollberger & Ehlert, 2016). With specific interest to this thesis, it is hoped that the use of FT_4:FT_3 and FT_4:rT_3 will clearly indicate the impact of stress, quantified by cortisol concentrations, on peripheral conversion of T_4. The reliability of these ratios is therefore compounded by the previously discussed reliability of the hormones involved (FT_4, FT_3 and rT_3), thus the greater within-day TE of both the FT_4:FT_3 and FT_4:rT_3 ratio could similarly reflect the circadian fluctuation in FT_3 (Abdalla & Bianco, 2014; Russell et al., 2008).

This study attempted to control for factors that could impact on thyroid function and therefore TH concentrations. However due to the interest in the natural daily fluctuations in TH concentrations, the study was conducted in free-living conditions to strengthen ecological validity. This in turn could impact on the variation in TH levels across time points and supports the argument against testing for thyroid dysfunction with one test in isolation of any other monitoring. It must be noted that the demands on the liver

approaching PHV might impact on peripheral conversion of T_4, however despite an even split of pre and post-PHV (n=6) in study two, there were no meaningful differences in typical error between these two groups. Although a relatively small sample size was used in study two (n=12), it was a homogenous population of males 12-15 y. It was outside the scope of this study to look at alternative populations, however future studies with larger sample sizes could use this method to assess LC-MS analysis for TH concretions within a broader population. However, the use of a specific male adolescent population would dictate that TH analysis by LC-MS is reliable in this population.

5.5 Conclusion

The results suggest that digital measures of BP and axillary BT are reproducible over a 24 hour period and can be useful tools when conducting research in an applied setting and as a monitoring method for adolescent males. However, researchers and practitioners must standardise test procedures in an attempt to control for some of the factors that introduce systematic and random variation. If consistent daily monitoring of BP and BT is undertaken, this should be recorded in similar conditions to RMR assessment. If this is not possible, then a standardised rest period should be applied prior to each measurement.

The results from this study also support the use of LC-MS in the analysis of THs. Despite the considerable random error noted with rT_3 and T_2 analysis in this study, the benefits of analysing the suite of TH is clear. The smaller volume of blood required allows for finger-tip capillary sampling as opposed to venepuncture, leading to more simplistic and less invasive sampling methods in children and adolescents (McNally et al., 2008). Importantly, the analysis of FT_3 by LC-MS had strong reproducibility between and within-days, due to FT_3 being the TH with greatest impact on metabolic rate and therefore health and athletic performance this is of paramount importance (Abdalla & Bianco, 2014). Finally, this study supports the inverse link between FT_3 and BP and BT in adolescent males. Therefore, frequent and consistent BT and BP measurements could be used in sports settings as surrogate markers for monitoring thyroid function in healthy adolescent males.

In the broader sense of this thesis, the combined approach of digital measures of BT and BP with TH quantification through LC-MS is deemed to be a reliable method of assessing thyroid function longitudinally in adolescent academy footballers. However, the typical errors reported here must be noted when interpreting the findings of subsequent longitudinal and intervention studies. Furthermore, the importance of individual biological variation in thyroid function quantified by FT_3 is clear, and therefore where possible, individual reference ranges should be noted at rest before inferring findings under different exercise and stress conditions.

Chapter 6:
The Impact of Varying Training Loads and Habitual Nutritional Intake on Thyroid Function in Adolescent Male Academy Football Players

6.1 Introduction

In chapters 4 and 5 of this thesis alternative methods of assessing thyroid function were evaluated. The results presented a strong case for using finger-tip capillary sampling methods for analysis of FT_4, FT_3, rT_3 and T_2 by LC-MS as a valid alternative to ELISA and ECLIA methods, in addition to its clear logistical advantages. Both studies evaluated alternative surrogates for when blood sampling is not possible and suggested BT and BP monitoring as potential alternatives. The results indicated both methods could be implemented as surrogate measures, specifically BT, if correct methodologies were used. However further evaluation of the relationship between clinical and subclinical thyroid dysfunction and these measures were needed. As such, these methods will be utilised, and subsequently further scrutinised, throughout this thesis when observing fluctuations in thyroid function in adolescent athletic populations due to TL, maturation and nutritional status.

With regard to thyroid function, stress has been noted to have detrimental effects on thyroid function, specifically in the reduction of T_4 and T_3 (Chatzitomaris et al., 2017; Chrousos & Gold, 1992). This is postulated to be due to the multi-functionality of the liver, with a low energy liver inhibiting T_4 conversion due to the prioritisation of other pathways, resulting in a host of health impairments including blood glucose maintenance, lipid metabolism and detoxification (Chiang, 2014; Mullur et al., 2014; Sendensky & Dufour, 2011). Stress is characterised by increased circulating adrenalin and cortisol, with an increase in circulating cortisol further inhibiting T_4 deionisation (Hackney & Saeidi, 2019; Límanová et al., 2009). This results in a detrimental cycle of poor metabolism and liver function (Kanaka-Gantenbein, 2005; Mullur et al., 2014; Tolfrey, 2016), which becomes increasingly likely during times of high stress (Mancini et al., 2016).

Adolescence presents special challenges, with an accumulation of stress factors associated with growth and maturation (Mostazir et al., 2016). Within athletic populations there is also additional factors that could potentially inhibit thyroid function, specifically T_3 concentrations (Kanaka-Gantenbein, 2005). These factors include but are not limited to the increased TL, and the significant EDs reported within adolescent male footballers (Briggs et al., 2015a; Morton, 2019; Naughton et al., 2016; Russell & Pennock, 2011),

furthermore, due to the high-intensity intermittent nature of football these implications could be further amplified (Hackney et al., 2012). Thus, within adolescent athletes the implications of growth, maturation, TL and nutritional intake can all result in greater demand and, if not met, impact on thyroid function and metabolic health (Mostazir et al., 2016). Accordingly, adolescent footballers could be at risk of ED related impairments in metabolism, with hypothyroidism possible, with associated implications on athletic performance, bone health, recovery and growth (Kanaka-Gantenbein, 2005).

Assessment of thyroid function across the football season within adolescent athletes would inform when individuals could be at risk of supressed thyroid function, and potentially give a greater insight into the endocrine response to RED-S. Therefore, the purpose of this study was to profile players' thyroid function in response to their habitual nutritional intake, TL and maturation status. This investigation aimed to identify any potential risk factors and accumulative 'hot-spots' within the season where thyroid function is most at risk, with a view to attenuating suppression of thyroid function with an acute-nutritional intervention in a subsequent study.

6.2 Methods

Using a cohort design, this longitudinal study aimed to assess thyroid function in a sample of adolescent male footballers under diverse exercising and stress conditions. Participants were monitored over a standard football season to examine the impact of maturation, TL and nutritional intake on TH, BT and BP. Data collection was completed between September and April of the 2018-2019 football season with three different test points identified. Test points were identified with the aim of gaining a thorough perspective of differing TL, thus subsequent variances in ED were evaluated. These test points are defined in Table 6.1.

Table 6.1 Test Condition Descriptors

Test Condition	Definition
Rest	A seven day period involving no formal academy training or fixtures
Training	A seven day period involving formal academy training but no fixtures
Fixture	A seven day period involving formal academy training and one competitive football fixture played

Where possible at least seven days separated each test condition, although not always feasible due to academy schedules, nonetheless it remained that at least 24-hours separated all conditions in all participants. During each testing condition, three days of data collection were undertaken assessing biological age, free-living EE, TL, EI, BT, BP and circulating THs. A schematic of the study design is outlined in Figure 6.1 with details of methods described below and in Chapter 3.

Ideally all data within each time point would have been collected in a controlled setting by the principal investigator, however on 13 occasions participants were unable to attend the 24 hour post exercise session. Therefore, on these occasions participants measured and recorded their own BP and BT using the equipment and methods outlined previously; blood sampling was disregarded in these circumstances. During the rest condition it was not possible to obtain a three day period in which participants could attend data collection sessions whilst not taking part in any formal training, therefore a baseline measurement was obtained for each participant following a period of over seven days without formal academy training.

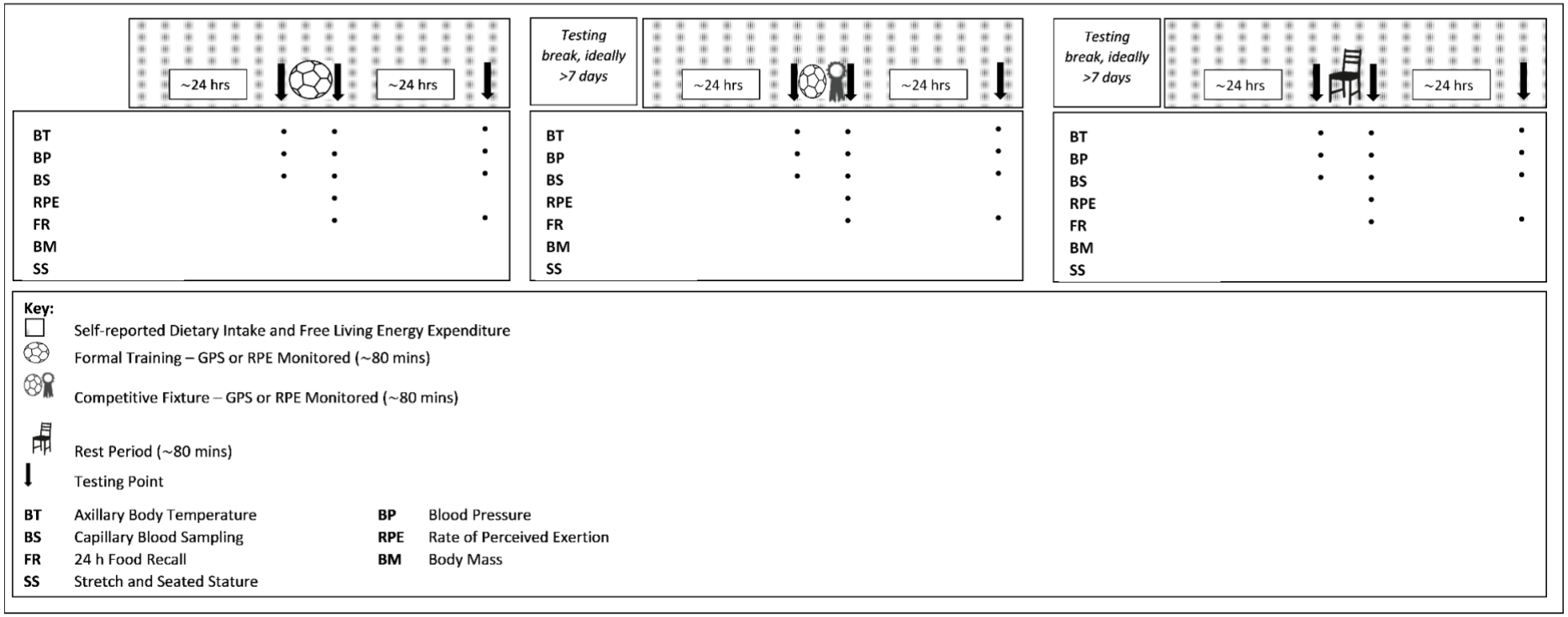

Figure 6.1 Schematic of Study Design.

6.2.1 Participants

Thirty adolescent males (14 ± 1 y) were recruited for this study from NUFC Academy, a Category One academy. Within the season of testing (2018-2019) all participants competed with the under 14, 15 and 16 squads. Inclusion criteria included a current member of NUFC Academy training at least three times per week, not injured at time of study commencement and with no known thyroid or liver disorders.

Prior to study commencement all participants and a parent/guardian attended an information event in which a verbal outline of the study including risks and benefits were given, any questions answered, participants were given information sheets and written informed consent was sought prior to the commencement of the study. In addition, all participants and an appropriate adult were shown how to operate all equipment (digital thermometers and BP monitors) for any occasions that any measurements were required to be taken away from the academy site. Understanding was checked by the principal investigator. Participants were also trained in how to correctly weigh food and complete their food diary (Livingstone et al., 1992) and were habituated with the two-pass 24-hour dietary recall method that would be used throughout the study (Ashley & Bove, 2003).

Sample size was estimated to provide 80% power to detect the smallest meaningful change at 0.05 level for circulating FT_3 concentrations (1.33 pmol/L), based on the measurement error study in Chapter 4. Consequently, it was estimated that 24 participants were required, thus 30 participants were recruited to allow for an anticipated 20% drop out rate. However, due to logistical issues in the testing environment, injuries and release of players, 13 participants withdrew prior to data collection resulting in 17 participants completing the study. However significant effects were found suggesting that the effect was larger than expected from the initial power calculation, or alternatively that variability was smaller, however further research could attempt to replicate these findings to ensure this was not an unlikely Type I error. Participant characteristics are outlined in Table 6.2. The Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria granted ethical approval and all participants gave parental written informed consent and their own assent prior to data collection.

Table 6.2 Participant Characteristics Dichotomised According to Playing Squad (Mean ± SD)

	Total	**U14**	**U15**	**U16**
	n=17	n=6	n=7	n=4
Age (y)	14.51 ± 0.76	13.75 ± 0.25	14.57 ± 0.35	15.56 ± 0.38
Body Mass (kg)	59.28 ± 13.27	47.60 ± 4.70	63.60 ± 14.41	69.25 ± 6.13
Stature (m)	1.69 ± 0.12	1.58 ± 0.03	1.71 ± 0.11	1.81 ± 0.06
BMI (kg/m^2)	20.57 ± 2.62	19.04 ± 1.23	21.55 ± 3.44	21.15 ± 1.77
Biological Age (y)	15.29 ± 1.83	13.37 ± 0.48	15.48 ± 0.78	17.83 ± 0.30

BMI, body mass index

6.2.2 Biological Age

Biological age was assessed on commencement of the study as described in section 3.2, using PHV as a measure of biological age (Mirwald et al., 2002).

6.2.3 Body Temperature and Blood Pressure

Both BT and BP were assessed via digital methods as described in sections 3.3 and 3.4 respectively. Measures were taken by the principal researcher with the exception of 13 instances in which the participants could not attend the academy site due to logistical reasons and therefore were responsible for measuring and recording their own results.

6.2.4 Blood Sampling

Blood sampling allowed the quantification of TH concentrations from ~500 µl of whole blood collected via fingertip-capillary sampling (providing ~200-300 µL of plasma for subsequent analysis), sampling and analysis was conducted as described in section 3.5.

6.2.5 Energy Expenditure

Energy expenditure was assessed in free-living conditions for three days during each test condition by tri-axial accelerometer (Actigraph GT3X, Florida) in all participants as outlined in section 3.7.1. Participants were instructed to wear the accelerometer from waking on the first day of testing and return when the final blood sample was taken.

During formal academy training and matches, EE was assessed via GPS monitoring (Catapult Optimeye S5, Catapult Sports, Australia) for the u15 and u16 age groups as outlined in section 3.7.2. The u14 squads exercising conditions could not be assessed via the same method, as such, rate of perceived exertion (RPE) was combined with session time (mins) to estimate session load. Although this does not allow for estimation of EE, it distinguishes subjective exercise intensities between test conditions.

6.2.6 Nutritional Intake

Nutritional intake was assessed for four days at each test point using a combined weighed food diary and 24-hour food recall method (Briggs et al., 2015b). Intakes were then analysed using commercially available software (Nutritics v5.097, Dublin) as outlined in Chapter 3.6.

6.2.7 Statistical Analysis

Statistical analysis was conducted using a custom-made spreadsheet (Microsoft Excel, Microsoft Corporation, Washington) and SPSS 24.0 (SPSS, Inc., Chicago, IL). For analysis of the repeated measures by group, a linear mixed model (LMM) was developed, with subsequent data presented as EMM ± SEM, data is presented in this format as is the convention with LMM approaches and represents the accurary of the model as an estimation of the true population effects. Linear mixed models have multiple advantages over ANOVA and ANCOVAs, including the ability to accommodate missing data points which were encountered due to the applied, free-living nature of this study, furthermore LMM can model nonlinear individual characteristics accommodating individual variations in baseline measurements and individual slopes. Each model was selected based on the lowest Schwarz's Bayesian Criterion, as an indicator of the best fitting model

(Drton & Plummer, 2017). All hormones were assessed using a random intercept model with fixed factors of maturation status, EE, EI, EA and carbohydrate intakes; respective baseline TH concentrations and subject were entered into each model as a random factor. Additionally, all post-hoc analyses were conducted using Sidak controlled group comparisons. For post-hoc analysis of EA, participants were categorised as previously outlined by Loucks et al. (2011) and highlighted in Table 2.4, maturation status by established thresholds outline in Table 3.1 (Mirwald et al., 2002) and carbohydrate intake as outlined in Table 6.3.

Table 6.3 Carbohydrate Intake Category Descriptors

Category	Carbohydrate Intake (g/kg/day)
1	< 3
2	3 – 4
3	> 4

6.3 Results

6.3.1 Nutritional Intake

The mean daily EI and macronutrient of players expressed with reference to TL is indicated in Table 6.4, with significant differences for EI ($F_{(2, 20} = 3.965$, $p = 0.035$) and protein ($F_{(2, 20)} = 7.562$, $p = 0.0.004$).

Mean daily EI and EE based on the training day is shown in Figure 6.2. A mean energy deficit was observed on all days, with a significant difference on match and training days.

Analysis of micronutrients relative to reference nutrient intake (RNI) values are shown in Figure 6.3. Mean intakes were greater than RNI for all micronutrients excluding calcium, selenium and iodine in 29, 29 and 41% of participants respectively.

Table 6.4 Total and Relative Energy and Macronutrient Intake of Academy Footballers by Training Day

	Rest	Training	Match	Mean
		Energy Intake*		
Per day (kcal)	2051 ± 402	1843 ± 220	1892 ± 421	1939 ± 370
Per unit BM (kcal/kg/day)	35.1 ± 13.9	35.9 ± 11.3	33.8 ± 11.1	35.6 ± 11.9
		Carbohydrate		
Per day (g)	249 ± 47	211 ± 35	229 ± 49	232 ± 47
Per unit BM (g/kg/day)	4.4 ± 1.4	4.2 ± 1.6	4.1 ± 1.3	4.2 ± 1.4
Energy contribution (%)	46.7 ± 11.3	42.8 ± 3.5	45.7 ± 4.4	45.3 ± 7.6
		Protein*		
Per day (g)	109 ± 26	87 ± 14	85 ± 19	95 ± 23
Per unit BM (g/kg/day)	1.9 ± 0.5	1.6 ± 0.3	1.5 ± 0.3	1.7 ± 0.4
Energy contribution (%)	21.9 ± 6.7	19.2 ± 3.9	18.3 ± 3.5	19.9 ± 5.2
		Fat		
Per day (g)	68 ± 30	64 ± 14	62 ± 22	64.6 ± 27.9
Per unit BM (g/kg/day)	1.3 ± 0.8	1.3 ± 0.5	1.1 ± 0.5	1.2 ± 0.6
Energy contribution (%)	29.9 ± 12.3	31.3 ± 5.4	28.9 ± 7.1	29.9 ± 8.9

*BM, body mass; mean ± SD, *; significance at p < 0.05 level*

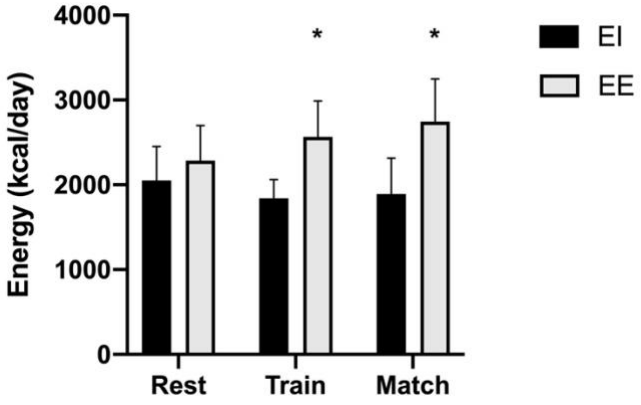

Figure 6.2 Energy Intake and Expenditure for Type of Training Day (Mean ± SD; *, significant difference at p < 0.05 level; EI, Energy Intake; EE, Energy Expenditure).

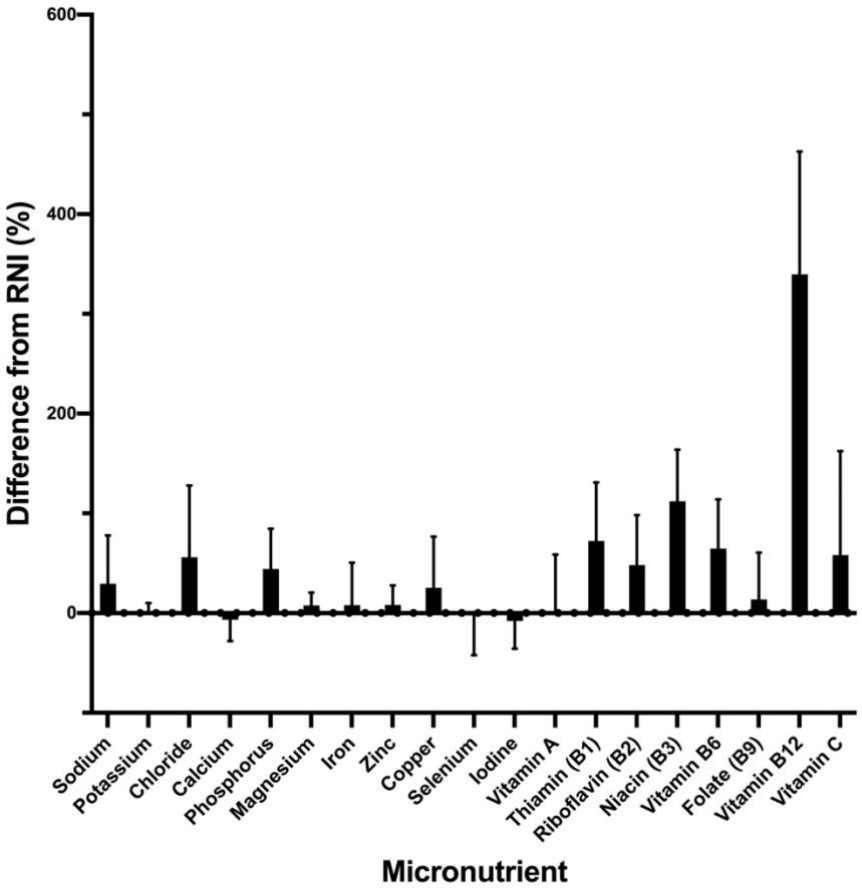

Figure 6.3 Micronutrient Composition of Academy Football Players, Relative to RNI Values (Mean ± SD; RNI, recommended nutrient intake)

6.3.2 Impact of Training

The findings from the LMM for all TH at all time points are presented in Figure 6.4. Although no findings reached statistical significance ($p > 0.05$), a trend was noted for decreased T_4 and T_3 24 hours post-match with a concurrent increase in rT_3.

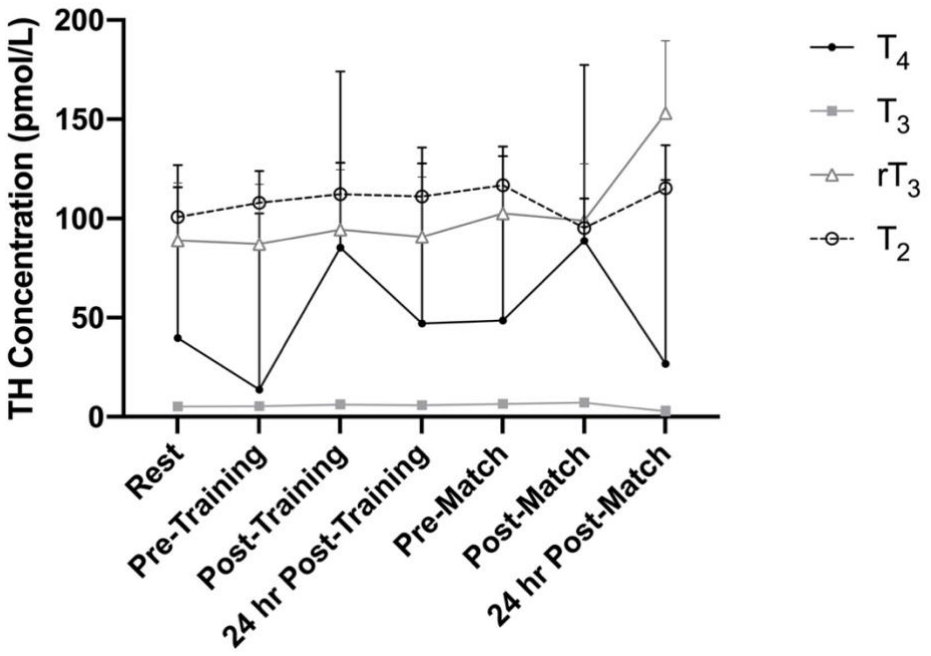

Figure 6.4 Changes in Mean Thyroid Hormone Concentrations Across Different Training Days in Academy Footballers (Estimated Marginal EMM ± SEM; n = 17).

6.3.3 Impact of Maturation

Maturation had a significant impact on T_4 ($F_{(9, 114} = 4.026$, $p = 0.015$) and T_3 ($F_{(6, 114} = 3.681$, $p = 0.002$) but for no other hormones. When categorised into established thresholds for maturity status (circa and post PHV), Table 3.1, post-hoc analyses identified those in circa PHV with lower T_4 and T_3 than those post-PHV (Figure 6.5); within this study no participants were pre-PHV.

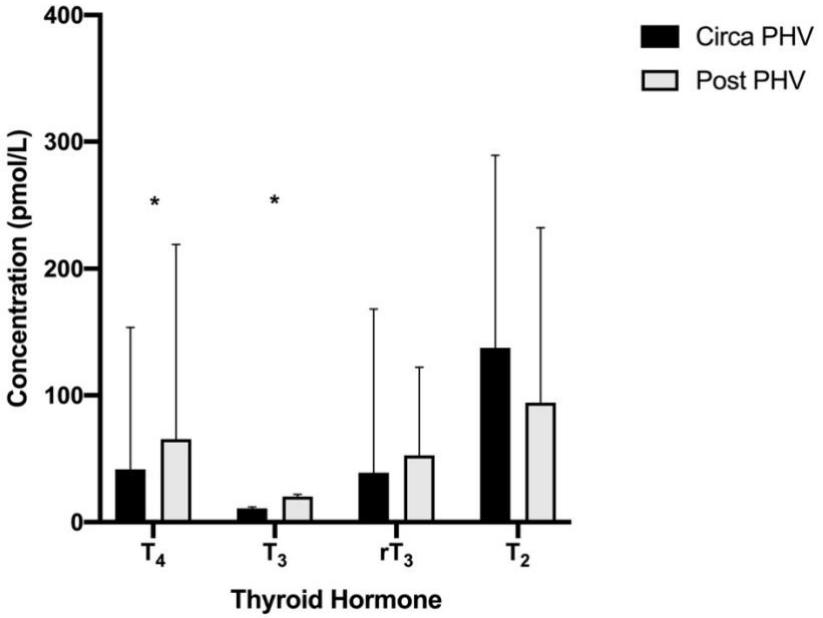

Figure 6.5 Impact of Maturation Status on Thyroid Hormone Concentrations in Adolescent Footballers. (EMM ± SEM; PHV, peak height velocity; *, significance at p < 0.05 level).

6.3.4 Energy Availability

Energy availability had a significant impact on T_3 ($F_{(2, 114} = 2.714$, $p = 0.02$) but no other TH. When categorised according to established thresholds (Loucks et al., 2011) (Table 2.4), mean weekly EA had a non-significant positive effect on all TH (Figure 6.6). Assessment of EA on different training days revealed no significant impact, however results indicated heightened T_3 and rT_3 on training days in participants with high EA (Figure 6.7). All participants were classified as low or suboptimal EA on match days. Although non-significant, results indicate alterations in TH concentrations between EA categories, particularly on training days.

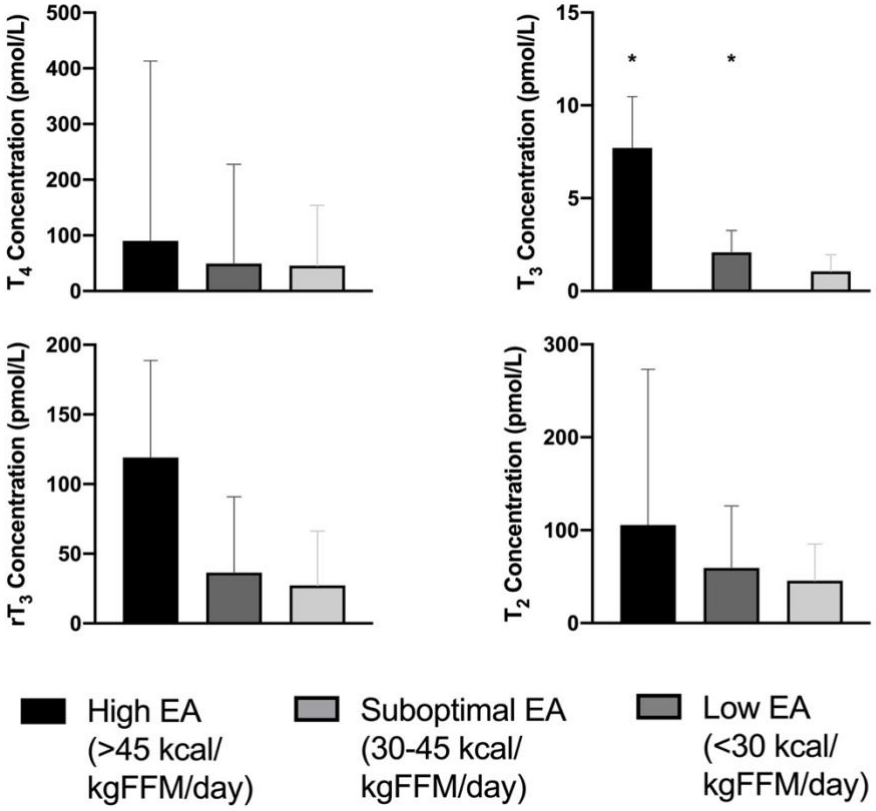

Figure 6.6 Mean Thyroid Hormone Concentrations by Energy Availability (EMM ± SEM; EA, energy availability; *, significance at p < 0.05 level).

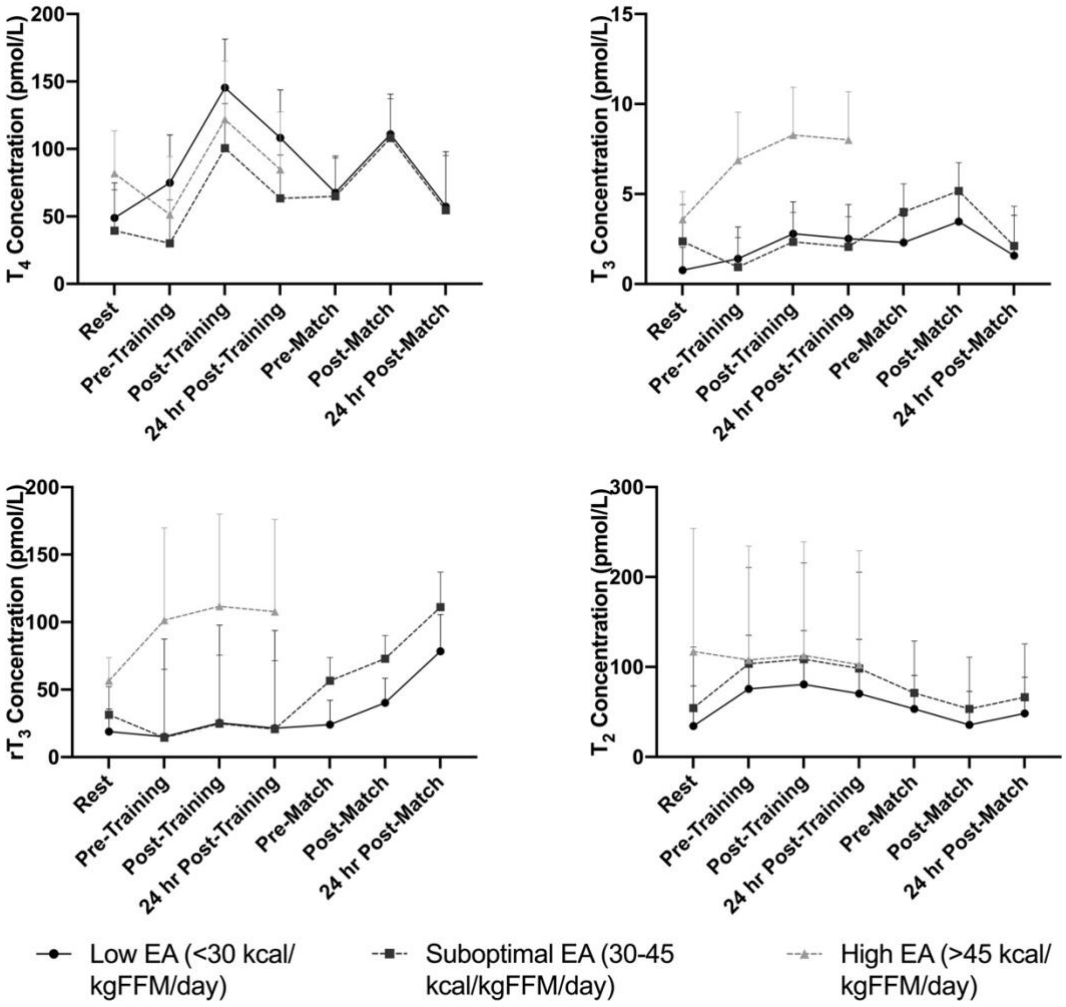

Figure 6.7 Thyroid Hormone Concentrations in Response to Football Training and Energy Availability in Adolescent Footballers (EMM ± SEM).

6.3.5 Impact of Carbohydrate Intake

Mean carbohydrate intake had no significant impact on any TH ($p > 0.05$), however there were modest positive associations between carbohydrate intake and T_4 ($r = 0.38$) and T_3 ($r = 0.49$) and small negative correlations between rT_3 and carbohydrate intake ($r = -0.0.26$) (Figure 6.8). Assessment of carbohydrate intake on different training days revealed a significant impact for T_4 ($F_{(2, 58} = 65.349$, $p = 0.001$), rT_3 ($F_{(2, 60} = 12.472$, $p = 0.001$) and T_2 ($F_{(2, 8} = 16.083$, $p = 0.002$) on training days (Figure 6.9).

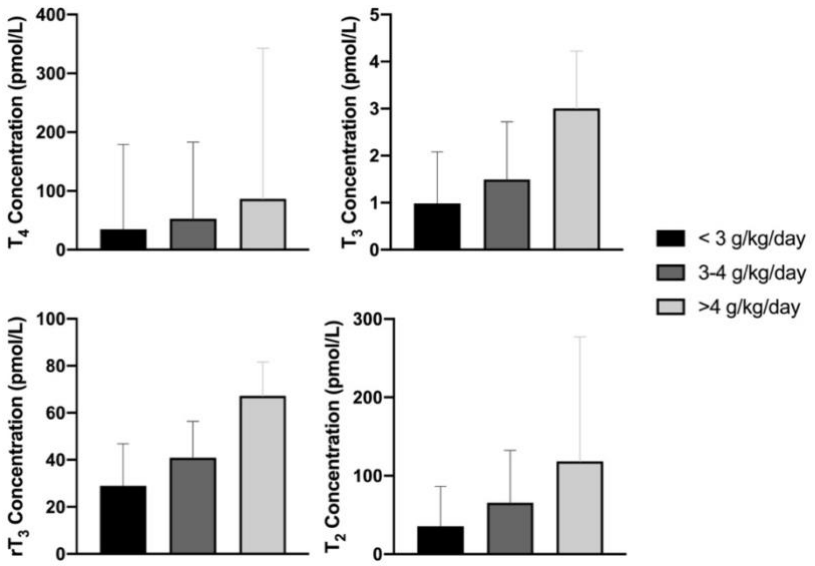

Figure 6.8 Thyroid Hormone Concentration by Carbohydrate Intake in Adolescent Footballers (EMM ± SEM).

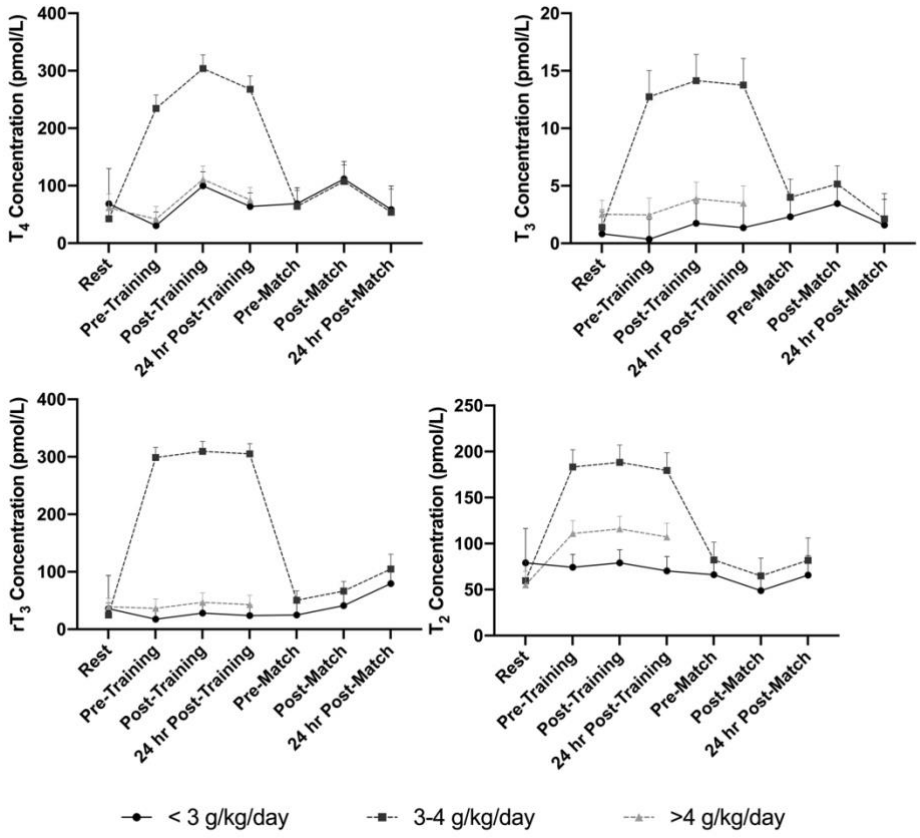

Figure 6.9 Thyroid Hormone Concentrations in Response to Football Training and Carbohydrate Intake in Adolescent Footballers (EMM ± SEM).

6.3.6 Body Temperature and Blood Pressure

No significant relationships were identified for the impact of maturation, EA, TL or carbohydrate intake on BT, SBP or DBP ($p > 0.05$ for all variables).

6.4 Discussion

The present study aimed to investigate the potential factors impacting on thyroid function in male adolescent football players. Accordingly, this study profiled thyroid function in response to maturation, EA, TL and nutritional intake in an attempt to identify periods at which thyroid function could be supressed. The data reveals that maturation status and EA have significant impacts on T_3 within adolescent male footballers, with indication that those in circa PHV and in LEA could be at risk of supressed T_3. Furthermore, findings suggest that carbohydrate intake has a significant effect on T_3 on a training day, but this was not reported on other days. Findings also imply that training day could impact on TH concentration with variation between rest, training and match days and a noted decline in T_4 and T_3 24 hours post-match. Together this data suggests that adolescents classified as circa-PHV and those with suboptimal or LEA could be at risk of supressed thyroid function, specifically low T_3.

6.4.1 Maturation

Maturation status had a significant impact on both T_4 and T_3 concentrations, with lower concentrations reported in those in circa compared to post-PHV. Furthermore, data from this study identified increased variation in TH concentrations across time points for those classified as circa rather than post-PHV. Within this study participants chronological age was 14 ± 1 y, however their biological age was 15.3 ± 1.8, reflective of the preference towards early and on-time maturers within professional soccer academies (Malina et al., 2017). Despite recruiting across three age categories, no pre-PHV individuals were recruited.

These findings are consistent with the decline in T_3 reported around pubertal growth (Rubenstein et al., 1973) and substantial changes in the HPT axis over childhood (Taylor et al., 2017). However, due to the lack of pre-PHV data, this study was unable to establish

if this is a decline or in fact solely an increase from circa to post-PHV. In contrast to earlier findings (Rubenstein et al., 1973) this study evidenced significantly lower T_4 as well as T_3 with no significant alterations in rT_3, indicating that this could be an alteration in TH status beyond that of peripheral conversion. Data from this study adds insight into more recent literature noting an approximately 110 kcal/day reduction in RMR from 10-15 years (Mostazir et al., 2016) and the negative correlation with maturation and REE (Sun et al., 2001). Due to logistical issues within this study, it was not possible to assess RMR alongside TH concentrations which could provide valuable insight and implications of this reduction in TH, particularly in T_3. As such, future research could endeavour to concurrently assess TH concentrations and RMR within adolescent athletes across different maturation statuses. The mechanism behind this reduction in T_4 and T_3 seen during peak maturation are unknown, however it is hypothesised to be an energy preservation mechanism to account for the additional energy demands of growth (Millward et al., 1976). As such, although the reduction in T_3 aligns with previous research, the reduction in T_4 is unexpected. It could be postulated that the alteration in this study compared to prior research could in part be due to the training status of the participants, with T_4 being lower in adult athletes compared to sedentary counterparts (Hawamdeh et al., 2012). However, this research has not been conducted in adolescent or paediatric populations and as such cannot be confirmed. These findings provide novel insight into the potential implications of maturation status on thyroid function in elite adolescent athletes and as such indicates that monitoring of thyroid function could be more important in those in circa-PHV than those post-PHV. Furthermore, this finding indicates the importance of closely monitoring other 'risk-factors' for suppressed thyroid function during PHV in an attempt to mediate and not further compound the suppression of T_4 and T_3 reported during this time.

6.4.2 Energy Availability

In agreement with existing literature (Heikura et al., 2017; Loucks et al., 1998; Loucks & Callister, 1993; Loucks & Heath, 1994; Loucks & Thuma, 2003), EA had a significant impact on mean T_3 with increased EA associated with increased T_3 at all time points. Furthermore, when EA was grouped according to established EA categories (Loucks et al., 2011), the data remained significant for T_3, with those in optimal EA having the highest concentrations followed by suboptimal and LEA categories, supporting the

concept of increased thyroid function with increased EA (Burke et al., 2018; Elliott-Sale et al., 2018; Loucks et al., 2011). Although not significant, findings from this study also indicated increased T_4 and rT_3 with increased EA. While the former supports the hypothesis of increased TH production and energy production under high EA, the increase in rT_3 contradicts this theory as rT_3 is often postulated to increase in LEA in energy preservation attempts (Boelen et al., 2008). However, due to the lack of statistical significance and greater typical error with rT_3 this should be considered carefully, and further research is required.

Specific match day EA indicated that all individuals were classified as in low or suboptimal EA for the 24 hours surrounding a match, with no participants classified as optimal or high. This has clear athletic performance-based implications (Burke et al., 2011) and the limited data for optimal and high EA must be considered when interpreting findings. However, data from the low and sub-optimal group provides an indication of slightly higher T_3 in sub-optimal compared to low pre- and post-match, whilst there were very limited differences in T_4 concentrations between groups. This initial data does suggest alterations in TH status on match day relevant to EA, with a more substantial impact on T_3 potentially signposting adaptations in peripheral conversion of TH, however more data are needed particularly in those with high or optimal EA. Data from the training day indicated increased T_3 in those with high EA compared to suboptimal and low, although not statistically significant this finding provides novel insight into the extent of the impact of EA on T_3 during football training and acknowledges the requirement for further research.

In this specific sample of adolescent male footballers, EI values were similar to those within literature when assessed via the same method and with similar significant differences in EI and EE on both match and training days (Briggs et al., 2015a; Russell & Pennock, 2011), but lower than those more recently found using DLW (Morton, 2019). Due to issues still remaining in EA within adolescent footballers, there is a clear need for increased nutrition provision and guidance. Furthermore, the data from this study begins to quantify the impact of LEA on T_3 and thus adds greater urgency to this increased

provision. In addition, the association between EA status and T_3 suggests that T_3 status could be used as a measure of EA status.

6.4.3 Training Load

Findings for this study found no significant differences between THs on match, training or rest days, contradicting previous literature of supressed T_3 up to 24 hours post PA (Chatzitomaris et al., 2017; Hackney & Saeidi, 2019; Kanaka-Gantenbein, 2005; Lambiase et al., 2012). However, despite not reaching statistical significance, there are indications of an impact of PA and TL on THs. Other confounding covariates including EA and maturation, small sample size and large individual responses could explain the lack of statistical significance.

The findings support supressed T_3 24 hours post-training and to a greater extent 24 hours post-match in line with previous research (Chatzitomaris et al., 2017; Hackney & Saeidi, 2019; Kanaka-Gantenbein, 2005; Lambiase et al., 2012). In addition, the concurrent increase in rT_3 24 hours post-match is expected and reflective of previous stress studies that have identified a negative correlation between T_3 and rT_3 12 hours post-interval exercise (Hackney et al., 2012). Together, these findings suggest an alteration in peripheral conversion of THs in an energy preservation attempt often seen in times of stress ED (Chatzitomaris et al., 2017; Fliers et al., 2015; Loucks & Callister, 1993; Michalaki et al., 2001). However, the decline in T_4 post-match is less expected and contradicts the theory of the TH response to PA being solely attributed to peripheral alteration and indicates instead an allostatic HPT response (Chatzitomaris et al., 2017). This reflects findings from three studies which similarly noted reductions in T_4 up to 24 hours after exercise, although not all significant (Beyleroglu, 2011; Hackney & Dobridge, 2009; Hackney et al., 2012). Although data do indicate a relationship between training load and THs, as already indicated, this is compounded by a significant impact of both EA and maturation. As such, the alterations in THs seen in response to training could in fact be due to the LEA reported on the same days as opposed to the stress of exercise itself.

Due to the lack of GPS provision to age groups within this study it is not possible to directly compare TL to TH concentrations. As such, although this study gives an indication of the impact of PA duration and subjective intensity, future research should look at conducting this research using exclusively GPS metrics to quantify and confirm TL. Although more data are required, these findings indicate that PA does impact on TH concentrations in adolescent footballers, with an indication that greater TLs have a larger impact on TH concentrations up to 24 hours post-exercise, particularly T_4, T_3 and rT_3, with implications for recovery and subsequent athletic performance.

6.4.4 Dietary Intake

Existing literature supports the importance of adequate carbohydrate intake for thyroid function (Kose et al., 2017; Molteberg et al., 2020), with indication that adequate carbohydrate intake can attenuate the consequences of LEA on thyroid function (Hackney & Dobridge, 2009; Kelly, 2000; Reinhardt et al., 1993; Serog et al., 1982). Data from this study supports this, with a trend towards increased mean TH concentrations with increased carbohydrate intake, however this was not significant. Nevertheless, independent assessment of the training-day time point revealed a significant interaction of carbohydrate intake on T_4, rT_3 and T_2 but not T_3. Contrary to current understanding, post-hoc analysis revealed that those in the middle category (3-4 g/kg/day) for carbohydrate intake had the greatest concentrations of TH, rather than the highest carbohydrate intake category (Kose et al., 2017; Molteberg et al., 2020). The mechanism behind this is unknown and could be postulated that another significant co-variate was having an impact on this such as maturation or EA. Furthermore, timing or type of carbohydrate could impact on the TH response to overall carbohydrate intake and as such this could be investigated in future research.

Despite the EE associated with match-play being greater than that of training, and having a more intermittent nature and, as such, increasing carbohydrate requirements (Burke et al., 2011), there was no significant finding or trend for carbohydrate intake on TH on a match-day. This could be due to the age of participants and thus a greater reliance on fat as a fuel compared to more mature and adult counterparts' lower endogenous carbohydrate stores and reduced glycolytic capabilities (Eriksson & Saltin, 1974;

Timmons et al., 2003, 2007). However, when analysed according to maturation status, there were no significant differences between groups. This could be due to the relatively high biological age within these participants and should be investigated in future research with those pre-PHV. Similar to the findings of EA, no individual fell into the highest category of intakes for carbohydrate (>4 g/kg/day) in the 24 hours surrounding the match, and accordingly were all under current recommended guidelines (Desbrow et al., 2014), which could confound results. However, the lack of association on a match day is likely due to the impact of EA on TH concentrations being more significant and as such dulling any impact that carbohydrate intake might have on TH concentrations. This finding could add more weight to the argument of optimal EA as a priority of thyroid function with the caveat that if EA is not below a certain point then carbohydrate intake could mitigate some of the negative impact of EA on thyroid function.

Findings from this study indicated that mean micronutrient intakes were below RNI values for calcium, selenium and iodine, contradicting previous findings that despite sub-optimal dietary fuelling practices micronutrient intakes are not adversely affected within adolescent male footballers in the UK (Naughton et al., 2017; Russell & Pennock, 2011). However, Russell and Pennock (2011) did indicate selenium intakes below RNI but this was not significant. Of particular reference to thyroid function is selenium and iodine intakes, with iodine essential for TH production and selenium as a cofactor for the deiodinase reactions (Canaris et al., 2000; Larson-Meyer & Gostas, 2020). However, post-hoc analysis of the impact of selenium and iodine intakes on THs were not conclusive within this study, potentially due to bigger confounding factors such as maturation and EA. Furthermore, the extent of this deficit is unknown due to limitations within the assessment of micronutrient intakes from dietary diary and recall methods and as such should be carefully considered. This finding does provide more current insight into the dietary intakes of adolescent male footballers and acknowledges that micronutrient intakes might be below RNI and that further research is required to determine the prevalence and extent of this. Furthermore, this has practical implications in the provision of nutrition support in this population and adds to the argument for gathering nutritional biomarkers in this population. With regard to this thesis, there was no clear impact of selenium and iodine intakes on TH concentrations beyond that of EA and maturation status.

6.4.5 Methodological Considerations

Although this study allowed free-living assessment of the impact of potential risk factors on thyroid function, the study design has inherent limitations. Firstly, the sample size available was constrained due to player availability within a Category One academy which resulted in only those circa or post-PHV being assessed, limiting the findings of the impact of maturation on thyroid function. In addition, despite the methods of dietary intake being valid within this population (Briggs et al., 2015b), it relies on accurate and honest responses from athletes which cannot be guaranteed, this has a clear impact particularly on micronutrient intakes as discussed. Future studies might choose to build on recent DLW studies in this population (Morton, 2019) to assess the impact of EA on thyroid function using gold standard methods. Furthermore, there were no opportunities to gather RMR data on these athletes due to time constraints and player availability, as such, although it is useful to collect TH data, it would be beneficial to correlate this with adaptations in RMR and assess the acute impacts as to date this has not been completed. The individual variation in baseline TH and response to stressor factors outlined in this study is large, reflected in large SD, and supportive of existing literature (Kapelari et al., 2008; Thienpont et al., 2013a). Accordingly, the SD should be taken into consideration alongside the TE scores noted in Chapter 5 when interpreting the findings. With respect to this, data from this study outlined that although EA, carbohydrate intake and maturation have an impact on thyroid function, there could be particular thresholds in which THs become most compromised. As such it would be beneficial to design a more controlled trial to assess the impact of multiple EDs and carbohydrate intake on THs to outline where thyroid function might become at risk. More controlled studies could also provide more valuable insight into if BT and BP could be utilised as surrogate markers for monitoring thyroid function, findings from this study were inconclusive and this could largely be due to the applied setting measures were taken in, including alterations in room and outdoor temperature, different clothing worn between measures, differences in distances covered between pitch and measurements; all of which can cause variation in measures and invalid results.

6.5 Conclusion

This study demonstrated that the key potential risk factors for supressed thyroid function in adolescent footballers are maturation, EA and carbohydrate intake. However, the extent to which this impacts on thyroid function requires further investigation, particularly when comparing EA >30 kcal/kg FFM/day and those in pre-PHV. These data suggest that adolescents undergoing PHV and in low or suboptimal EA could be at risk of supressed thyroid function, particularly T_3, and specifically in the 24 hours following a match. Furthermore, the findings suggest that carbohydrate intake could impact on TH concentration, with indications that higher intakes have a positive correlation with T_3, however noting that this might not always mitigate the consequence of LEA, particularly if the EA is below a certain level. However, this has not been quantified. Accordingly, these findings indicate that adequate EA is of paramount importance for thyroid function in adolescent footballers and that nutritional support, adequate EA and carbohydrate intake could mitigate the decline in T_3 seen in response to competitive match-play.

Chapter 7:
The Acute Impact of Fructose and Glucose Co-ingestion on Thyroid Function Following Training and Competitive Fixtures in Adolescent Male Football Players

7.1 Introduction

Previous findings have indicated that multiple stress-factors within adolescent footballers can impact on thyroid function, with maturation, EA, carbohydrate intake and TL having the greatest impact. Within the elite academy environment, the training frequency and demands increase when players begin full-time within the academy, typically when they enter the u17 age category and become full-time scholar athletes, with evidenced increased TD and HID with each age category from u11 to u18s (Buchheit et al., 2010). As such, despite most athletes already having undergone PHV at this stage, it could be understood that the multiple physical stress factors and concurrent psychological stress of being a paid professional athlete could cause further detriments to thyroid function, most notably through poor EA.

Due to the multi-functionality of the liver, a low energy liver can inhibit T_4 conversion due to the prioritisation of other pathways, resulting in a host of health impairments including blood glucose maintenance, lipid metabolism and detoxification (Chiang, 2014; Mullur et al., 2014; Sendensky & Dufour, 2011). Stress is characterised by increased circulating adrenalin and cortisol, however, increased circulating cortisol inhibits T_4 deionisation further (Hackney & Saeidi, 2019; Límanová et al., 2009). This results in a detrimental cycle of poor metabolism and liver function (Kanaka-Gantenbein, 2005; Mullur et al., 2014; Tolfrey, 2016), which becomes increasingly likely during times of high stress (Mancini et al., 2016). Within sporting populations there is an additional factor impacting on T_3 levels and therefore metabolic health due to increased TL, amplified if high-intensity intermittent exercise is undertaken (Hackney et al., 2012). Furthermore, significant EDs have been reported within adolescent male academy footballers (Briggs et al., 2015a; Morton, 2019; Russell & Pennock, 2011) and outlined in Chapter 6. Findings from Chapter 6 identified supressed T_4 and T_3 concentrations immediately following and up to 24 hrs after competitive fixtures, during high TLs and when in LEA status. This is indicative of bouts of short-term ED related impairments in metabolism with acute bouts of hypothyroidism apparent at greater EDs, with potential implications for football performance and health, including decreased neuromuscular performance (Mountjoy et al., 2014; Tornberg et al., 2017).

Although alterations in T_4 and T_3 were apparent, the extent and implications of reductions in circulating T_3 are of greatest importance, indicting poor peripheral deiodination of T_4 by D2. It is postulated that this could be an energy preservation mechanism as this response is more prominent when in LEA. Moreover, Chapter 6 identified that carbohydrate intake had an impact on T_4 and T_3 with increased intakes associated with greater circulating concentrations. Therefore, due to the high intensity intermittent nature of football and the role of the liver in TH conversion, T_3 suppression is likely in response to both low EA and low liver glycogen due to a combination of adrenal-accelerated glycolysis, increased Cori cycle activity and increased cortisol inhibiting TH conversion. Consequently, this could be counteracted by an exogenous carbohydrate load (McAllister et al., 2016) that could in turn restore thyroid function. Thus, it is proposed that a carbohydrate load post-exercise could not only improve EA but could rapidly replenish liver glycogen and attenuate the stress response, attenuating the consequences of intense exercise, poor EA and low carbohydrate intake on T_3 concentration.

The co-ingestion of fructose and glucose (sucrose) is noted to replenish liver glycogen stores more efficiently than the two monosaccharides alone (Fuchs et al., 2016a; Gonzalez et al., 2017b). Whilst glucose is preferentially metabolised by extra-splanchnic tissues, fructose is primarily metabolised within the liver after being transported into the enterocyte by the non-sodium dependant GLUT5 transporter whilst glucose relies mainly on the sodium-dependant glucose transporter 1 (SGLT1) (Gonzalez & Betts, 2018). Thus, due to the utilisation of different intestinal transporters (GLUT2, GLUT5, GLUT12 and SGLT1) the co-ingestion of glucose and fructose can accelerate the delivery into circulation and subsequently accelerate uptake by the liver by SGLT1 and GLUT2 for glucose and fructose respectively. Ultimately allowing for increased absorption and rapid liver glycogen restoration whilst also limiting gastro-intestinal distress and increasing total EI (Fuchs et al., 2016b; Gonzalez et al., 2017b). Furthermore, due to glucose stimulating insulin secretion from the pancreatic β cells, if high intakes of glucose are ingested, rebound hypoglycaemia is likely to occur resulting in subsequent stress responses. In contrast, fructose does not stimulate insulin secretion and therefore the combined ingestion should rapidly replenish liver and blood sugar without the risk of rebound hypoglycaemia and further stress reactions (Elliott et al., 2002). Therefore, it is hypothesised that the ingestion of a sucrose bolus following a football match or heavy

training will replenish liver glycogen and in turn restore normal deiodination of T_4 and thus thyroid function with a subsequent positive effect on systemic health, recovery and future sporting performance.

Therefore, the purpose of this study was to investigate the acute impact of fructose and glucose co-ingestion following a competitive fixture and heavy-training session on thyroid function and cortisol during a standard in-season week in male adolescent football players.

7.2 Materials and Methods

7.2.1 Study Design

Using a randomised blinded crossover design, this study aimed to assess the impact of fructose and glucose co-ingestion on thyroid function following heavy training sessions and competitive fixtures in a sample of elite male football players. Data collection was completed between September and December of the 2019-2020 football season with two seven day periods identified to reflect a standard in-season week, each including five training days, one competitive fixture and a recovery day. An example weekly schedule is indicated in Figure 7.1. Throughout each seven-day test point, EI, EE, TL, TH, cortisol, BT and BP were assessed with methods described below and within General Methods (Chapter 3). A schematic outlining the experimental process is depicted in Figure 7.2. Ideally two samples would have been collected daily, however, to ensure compliance and limit potential dropouts in the latter parts of the week, a decision was made to eliminate the two sampling sessions immediately post training on Thursday and the 24 hr follow up. This allowed the participants a ~24 hr break from sampling.

Chapter 6 identified that thyroid function might be at risk following a competitive fixture. Therefore, a nutritional intervention was implemented on this day. Furthermore, within this specific squad of players, TL on an endurance-based day (frequently day MD -3) is similar and sometimes greater than a MD with perceived wellness the following day substantially lowered. In turn, it was decided to also implement the nutritional intervention following this training session.

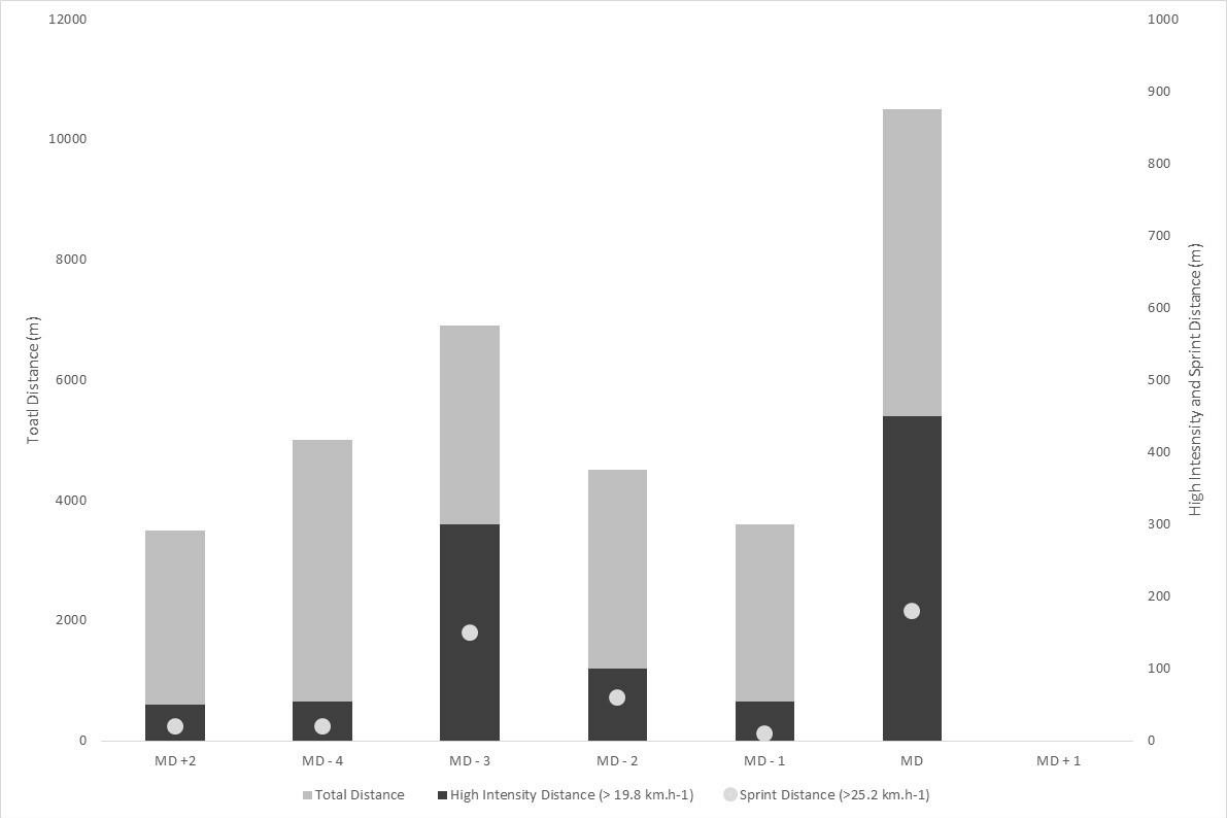

Figure 7.1 Weekly Training Structure for Total Distance, High Intensity Distance and Sprint Distance in u18 Outfield Players (MD, match day)

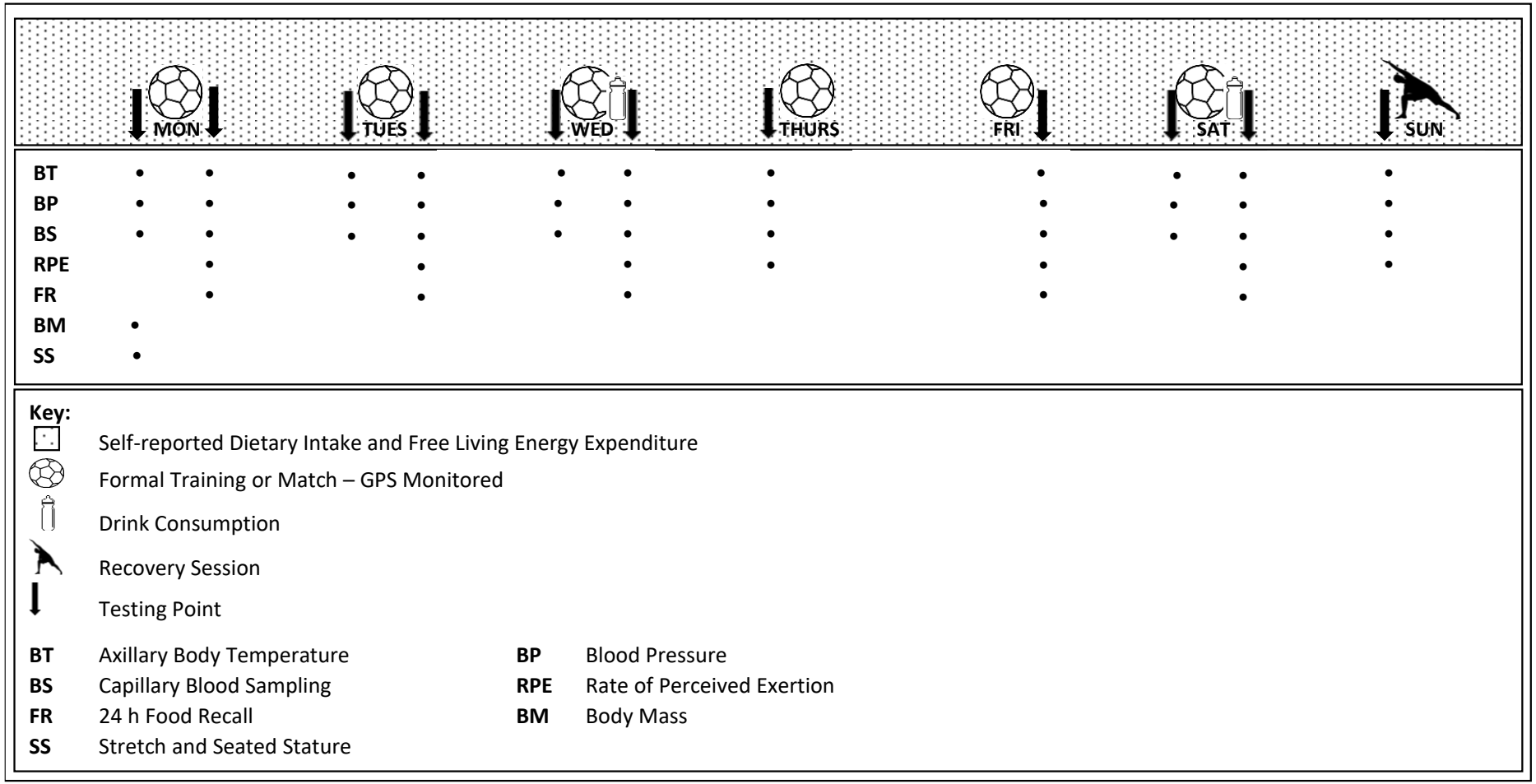

Figure 7.2 Schematic Overview of Study Design.

7.2.2 Participants

In total, 16 adolescent males (17 ± 0 y) were recruited from NUFC Academy, a Category One academy. Within the football season of testing (2019-2020) all participants were part of the under 18 squad and therefore were full-time scholars within the academy. Inclusion criteria included a current squad member in full training, not injured or banned at time of study commencement and with no known thyroid or liver disorders.

Sample size assessment was computed to determine 80% power to detect the smallest meaningful change at 0.05 level for circulating FT_3 concentrations (1.33 pmol/L), based on the previous measurement error study (Chapter 5). Consequently, it was estimated that 12 participants were required, thus all 16 registered outfield players were recruited prior to study commencement to account for a 25% drop out rate. Due to injury, unavailability and international duties, four players were unavailable during the period of testing and therefore 12 participants completed the study (17 ± 0 y). Participant details by test week are detailed in Table 7.1. Ideally, test weeks would have been completed within close proximity. However due to training, fixture schedules and player availability, there were 10 weeks between test sessions. The Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria granted ethical approval and all participants gave parental written informed consent prior to data collection.

Prior to study commencement, all participants and coaches or staff impacted by testing attended an information event in which a verbal outline of the study including risks and benefits were given and any questions answered. Participants were given information sheets and written informed consent was sought prior to the commencement of the study. Participants were also trained on how to correctly weigh food and complete their food diary (Livingstone et al., 1992) and were habituated with the two-pass 24-hour dietary recall method which would be used throughout the study (Ashley & Bove, 2003). To enhance compliance, weekly schedules were adjusted and given both physically and digitally to all players involved with testing. During testing weeks, players were digitally reminded about the daily testing procedures.

Table 7.1 Participant Characteristics by Test Period

	Week One	**Week Two**
	n = 12	n =10
	Mean ± SD	Mean ± SD
Age (y)	17 ± 0	17 ± 0
Body Mass (kg)	70.32 ± 6.60	72.38 ± 6.89
Stature (m)	1.77 ± 0.35	1.79 ± 0.45
BMI (kg/m^2)	22.30 ± 1.63	22.60 ± 1.20
Sum of Four Skinfolds (mm)	32.59 ± 7.17	32.36 ± 7.90
Body fat (%)	10.05 ± 7.17	10.21 ± 1.12

BMI, body mass index; SD, standard deviaton

7.2.3 Biological Age and Anthropometric Assessment

Biological age was assessed on commencement of the study as described in section 3.2, using PHV as a measure of biological age (Mirwald et al., 2002). The maturing offset was 1.9 ± 0.5 y beyond PHV indicating that all players were in a positive maturity offset and thus were of a similar maturation status. Anthropometric assessments of players were conducted on day one of each testing week, using the four site equation outlined in section 3.2 to predict body fat (%) (Reilly et al., 2009).

7.2.4 Body Temperature and Blood Pressure

Both BT and BP were assessed via digital methods as described in section 3.3 and 3.4 respectively. Measures were taken at all time points outlined and were taken by the principal researcher on all occasions.

7.2.5 Blood Sampling

Blood sampling allowed the quantification of TH concentrations (T_4, T_3, rT_3 and T_2) and cortisol from ~500 µl of whole blood collected via fingertip-capillary sampling (providing ~200-300 µL of plasma for subsequent analysis). Sampling and analysis was conducted as described in section 3.5.

7.2.6 Energy Expenditure

Energy expenditure (EE) was assessed in free-living conditions in all participants for each seven-day testing period by tri-axial accelerometer (Actigraph GT3X, Florida) as outlined in section 3.7. Participants were instructed to wear the accelerometer from waking on the first day of testing and return when the final blood sample was taken. Participants were advised to remove the accelerometer during matches and pitch-based training and were encouraged to wear during showering and sleeping but were allowed to remove if this caused discomfort. Compliance was checked through wear-time analysis (ActiLife, Florida). During formal academy training and matches, EE was assessed via GPS monitoring (Catapult Optimeye S5, Catapult Sports, Australia) for all players as outlined in section 3.7. In addition, session RPE was combined with session time (mins) to estimate session load.

7.2.7 Nutritional Intake

Nutritional intake was assessed for seven days at each test period using a combined weighed food diary and 24-hour food recall method (Briggs et al., 2015b). Intakes were then analysed using commercially available software (Nutritics v5.097, Dublin) as outlined in section 3.6.

7.2.8 Nutritional Intervention

Following the heavy endurance day (MD -3) and the competitive fixture, participants consumed either a glucose and fructose beverage or a placebo. This drink was offered to players on cessation of activity and approximately 15 mins before post-session assessments were undertaken. Subjects received a drink volume of 3.33 ml/kg giving a dose of 1.2 g/kg of sugar beet derived sucrose (Silver Spoon, Peterborough, United Kingdom) and 20 mmol/l NaCl (Sainsburys, London, United Kingdom) (Fuchs et al., 2016a) with the addition of 0.8 g/kg ml no added sugar orange cordial (Sainsburys, London, United Kingdom), providing 318.6 ± 29.0 kcal of energy. Similarly, the placebo 3.33 ml/kg of fluid contained 0.8 g/kg no added sugar orange cordial and 20 mmol/l NaCl. Both drinks were volume matched and taste matched as closely as possible, but due to the nature of the study, were not isocaloric.

7.2.9 Statistical Analysis

Statistical analysis was conducted using a custom-made spreadsheet (Microsoft Excel, Microsoft Corporation, Washington) and SPSS 24.0 (SPSS, Inc., Chicago, IL). Descriptive data were calculated as mean ± SD and differences between test weeks were assessed by two-way ANOVA. For analysis of the repeated measures by treatment group, a linear mixed model (LMM) was developed, with advantages as outlined in Chapter 6. Each model was selected based on the lowest Schwarz's Bayesian Criterion, as an indicator of the best fitting model (Drton & Plummer, 2017). All hormones were assessed using a random intercept model with fixed factors of treatment (placebo, carbohydrate intervention) and time as main and interaction effects. Respective baseline hormone concentrations and ED (weekly, match and heavy training day) were entered into each model as a covariate and subject was entered as a random factor into each model. Additionally, all post-hoc analyses were conducted using Sidak controlled group comparisons. For post-hoc analysis of carbohydrate intake, participants were categorised into four equal groups as described in Table 7.2; for further post-hoc analysis of EA, EA was categorised as previously outlined by Loucks et al. (2011) and highlighted in Table 2.4.

Table 7.2 Carbohydrate Intake Category Descriptors

Category	Carbohydrate Intake (g/kg/day)		
	Mean	**Match Day**	**Heavy Training**
1	< 3.198	<3.822	< 3.034
2	3.198 - 3.804	3.882 – 4.910	3.034 – 4.400
3	3.804 – 4.110	4.910 – 6.339	4.400 – 5.136
4	> 4.110	> 6.339	> 5.136

7.3 Results

7.3.1 Energy Intake and Macronutrients

The mean daily EI and macronutrients were not significantly different between testing bouts (Table 7.3). The ANOVA revealed no significant effect for EI ($F_{(1,7)} = 0.061$, $p = 0.811$), carbohydrate ($F_{(1,7)} = 0.206$, $p = 0.664$), protein ($F_{(1,7)} = 0.05$, $p = 0.974$) or fat ($F_{(1,7)} = 0.299$, $p = 0.602$) intakes across the two testing weeks.

Table 7.3 Energy and Macronutrient Intakes by Test Week

	Week One	**Week Two**
	n = 12	n =10
	Mean ± SD	Mean ± SD
Energy Intake (kcal/day)	2285.96 ± 282.41	2311.03 ± 488.96
Energy Intake (kcal/kg/day)	33.12 ± 4.46	32.95 ± 7.70
Carbohydrate Intake (g/day)	252.69 ± 31.92	259.93 ± 65.77
Carbohydrate Intake (g/kg/day)	3.75 ± 0.61	3.78 ± 1.00
Protein Intake (g/day)	137.16 ± 23.18	136.66 ± 23.29
Protein Intake (g/kg/day)	1.91 ± 0.27	1.93 ± 0.40
Fat Intake (g/day)	80.61 ± 11.46	78.11 ± 20.22
Fat Intake (g/kg/day)	1.16 ± 0.20	1.10 ± 0.30

The mean daily EI of players expressed with reference to TL is indicated in Table 7.4. There were no significant differences between days for EI ($F_{(1, 15)} = 0.345$, $p = 0.625$), carbohydrate ($F_{(1, 15)} = 0.530$, $p = 0.596$), protein ($F_{(2, 22)} = 2.697$, $p = 0.090$) and fat ($F_{(2, 22)} = 0.196$, $p = 0.824$).

Table 7.4 Total and Relative Energy and Macronutrient Intake of Academy Football Players Categorised by Training Day

	Light	Heavy	Match	Mean
		Energy Intake		
Per day (kcal)	2484 ± 474	2241 ± 756	2360 ± 688	2362 ± 285
Per unit BM (kcal/kg/day)	35.7 ± 7.8	31.9 ± 10.6	33.8 ± 9.8	33.8 ± 4.5
		Carbohydrate		
Per day (g)	276 ± 68	247 ± 83	286 ± 99	270 ± 31
Per unit BM (g/kg/day)	3.9 ± 1.0	3.5 ± 1.2	4.1 ± 1.5	3.9 ± 0.5
Energy contribution (%)	42 ± 6	42 ± 7	45 ± 4	43 ± 3
		Protein		
Per day (g)	148 ± 35	132 ± 48	110 ± 31	130 ± 21
Per unit BM (g/kg/day)	2.1 ± 0.6	1.9 ± 0.6	1.6 ± 0.4	1.9 ± 0.3
Energy contribution (%)	24 ± 3	24 ± 5	19 ± 5	22 ± 2
		Fat		
Per day (g)	88 ± 21	81 ± 34	86 ± 25	85 ± 15
Per unit BM (g/kg/day)	1.3 ± 0.3	1.2 ± 0.5	1.2 ± 0.3	1.2 ± 0.2
Energy contribution (%)	32 ± 5	31 ± 6	33 ± 3	32 ± 3

BM, body mass; mean ± SD; n=12, values do not include intervention beverage

Analysis of micronutrients relative to reference nutrient intake (RNI) values are shown in Figure 7.3. Mean intakes were greater than RNI for all micronutrients excluding potassium and calcium, of these two nutrients, five and four players were below RNI for each respective nutrient.

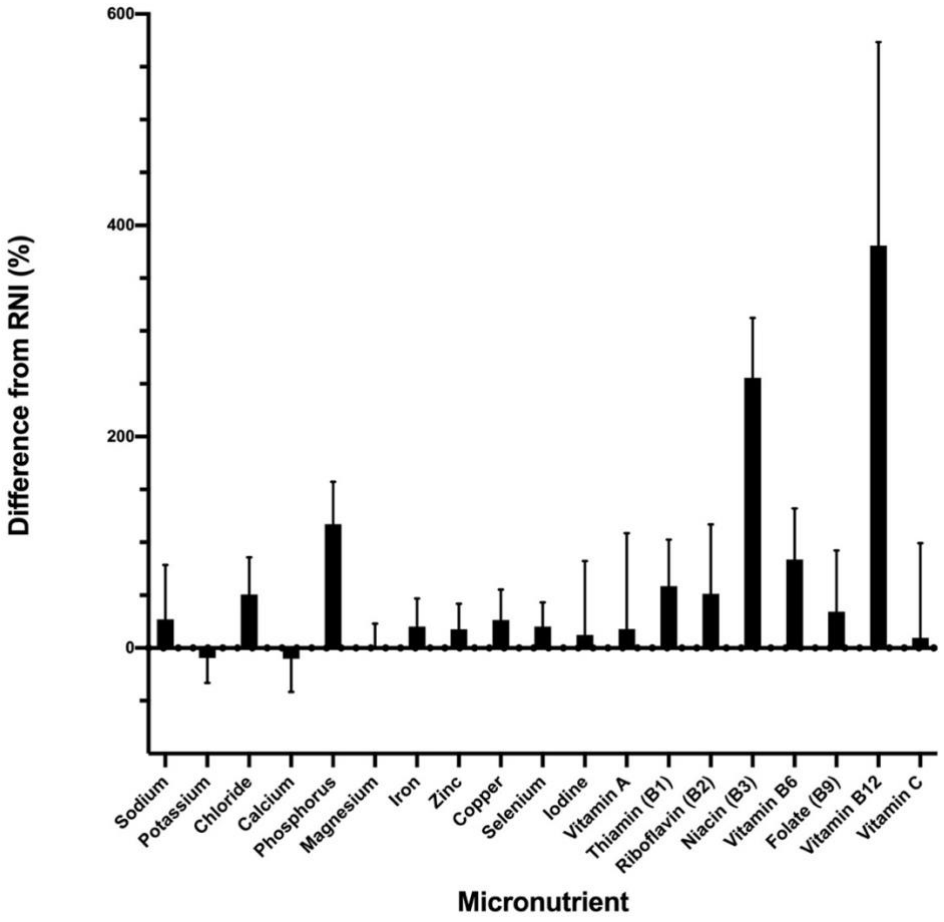

Figure 7.3 Micronutrient Consumption of Academy Football Players Relative to RNI Values. (Mean ± SD; RNI, recommended nutrient intake)

7.3.2 Energy Expenditure and GPS Metrics

Free living EE assessed via accelerometery did not differ significantly between test week one (2735 ± 529 kcal/day) and week two (2895 ± 482 kcal/day) ($F_{(1, 7)} = 0.850$, $p = 0.387$). Similarly, there were no significant differences for total additional EE from training and match activities between week one (2482 ± 549 kcal/week) and week two (2377 ± 596 kcal/week), $F_{(1, 79)} = 0.421$, $p = 0.533$). When assessing differences between specific training days on different test weeks, no significant differences for player load (PL) were found for moderate training ($F_{(1, 6)} = 0.166$, $p = 0.698$), heavy training($F_{(1, 7)} = 1.409$, $p = 0.274$) or competitive fixture ($F_{(1, 7)} = 0.391$, $p = 0.552$). Mean group differences across moderate, high and match sessions were significantly different for total distance (TD), high intensity distance (HID), very high-speed running (VHSR), PL and time above MAS (t > MAS) (Figure 7.4), indicating a manipulation in training demands and higher energy cost of match, compared to heavy and moderate training.

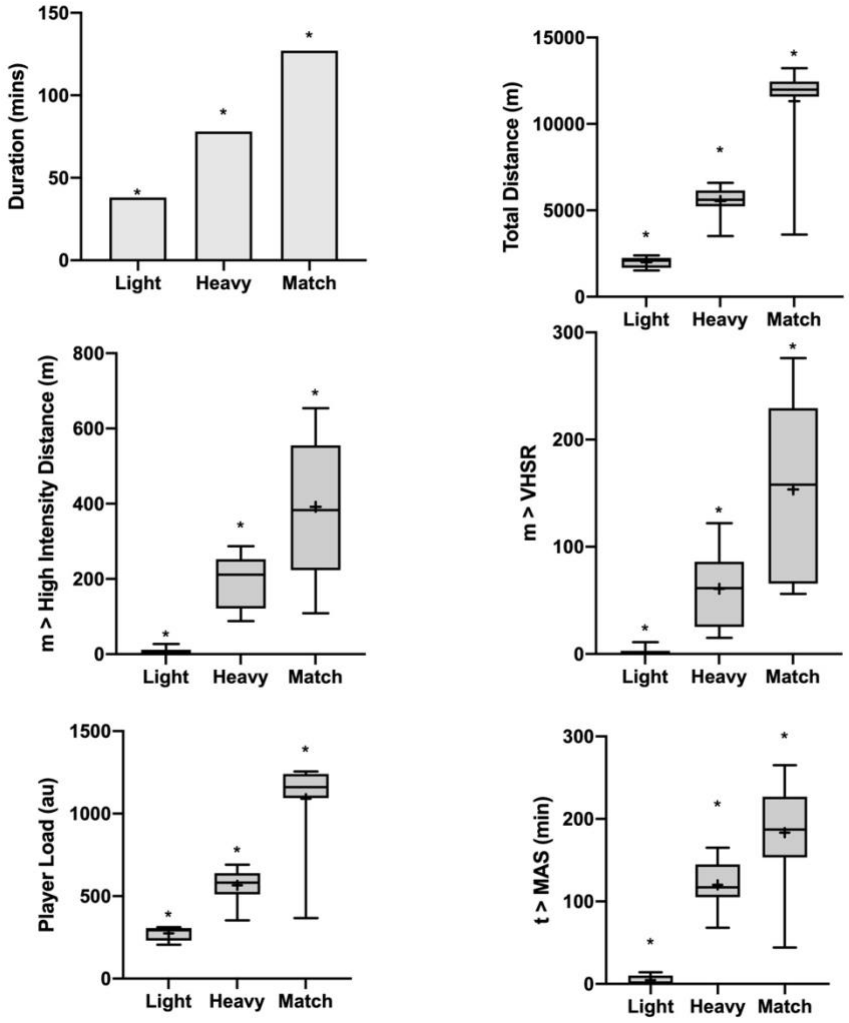

Figure 7.4 Training and Match Load for Duration, Total Distance, m>HID, m>VHSR, Player Load and t>MAS. Box and whisker plots with median values, interquartile ranges, minimum and maximum values (+; mean value, *; significance at p<0.05 level).

7.3.3 Energy Balance

Mean daily EI and EE based on the training day are shown in Figure 7.5. A mean energy deficit was observed on all days, with a significant difference on MD and heavy training days. The addition of the carbohydrate supplement following heavy training and the competitive match increased overall daily EI (1296 ± 129 kcal) and carbohydrate (324 ± 32 g), however failed to fully counteract the ED.

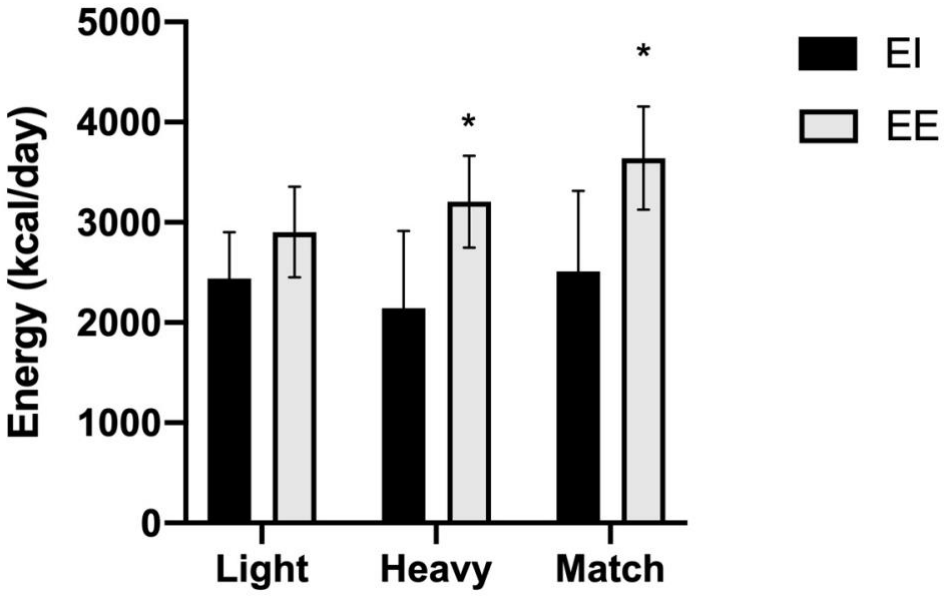

Figure 7.5 Energy Intake Compared to Energy Expenditure for Type of Training (Mean ± SD; EI, energy intake; EE, energy expenditure; *, significant difference at p < 0.05 level).

7.3.4 Thyroid Hormones

The findings from the LMM for all TH at all time points are presented within Table 7.5, with significant main effects of treatment and treatment*time interactions highlighted in bold. All post-hoc analyses reported are Sidak corrected comparisons.

Table 7.5 Estimated Marginal Means and Effects of Time and Carbohydrate Treatment on Thyroid Hormone Concentrations Across a Competitive Football Week

Hormone	Time	Treatment				Main Effects			
		Intervention		Placebo		Treatment		Treatment*Time	
		Mean	SE	Mean	SE	*F*	*P*	*F*	*P*
T_4 (pmol/L)	Baseline[a]	9.497	1.420	11.888	2.030				
	Mon Post	**7.190**	**1.492**	**13.616**	**1.973**			**8.104**	**0.005**
	Tue Pre	8.893	1.592	8.666	1.977			0.000	0.990
	Tue Post	11.966	1.470	11.804	1.984			0.007	0.932
	Wed Pre	10.896	1.397	11.078	1.811			0.008	0.930
	Wed Post	9.803	1.670	13.704	1.986	**5.026**	**0.027**	2.473	0.100
	Thurs Pre	8.950	1.558	10.259	1.811			0.364	0547
	Fri Post	11.860	1.474	9.671	2.201			0.826	0.365
	Sat Pre	12.254	1.477	15.651	2.221			1.948	0.165
	Sat Post	12.307	1.559	15.811	1.999			2.300	0.132
	Sun Pre	11.486	1.694	13.380	1.999			0.615	0.434
	Mean*	**10.564**	**0.460**	**12.384**	**0.616**				
T_3 (pmol/L)	Baseline[a]	17.063	2.066	12.093	3.456				
	Mon Post	12.051	4.962	8.165	6.068			0.277	0.600
	Tue Pre	4.090	5.040	12.960	6.106			1.34	0.249
	Tue Post	10.142	4.837	8.735	6.154			0.037	0.848
	Wed Pre	12.898	4.641	10.957	5.599			0.081	0.777
	Wed Post	12.133	5.453	7.330	6.162	**6.630**	**0.011**	0.388	0.535
	Thurs Pre	8.653	5.060	12.186	5.682			0.246	0.621
	Fri Post	10.710	4.881	13.300	6.834			0.109	0.742
	Sat Pre*	**27.941**	**4.859**	**49.799**	**6.760**			**7.606**	**0.007**
	Sat Post*	**26.265**	**5.025**	**46.787**	**6.203**			**7.252**	**0.008**
	Sun Pre*	**26.620**	**65.460**	**47.590**	**6.216**			**7.505**	**0.007**
	Mean*	**15.114**	**2.535**	**21.781**	**2.553**				
rT_3 (pmol/L)	Baseline[a]	66.303	7.784	46.239	14.717				
	Mon Post	51.141	9.268	63.199	11.236			0.675	0.413
	Tue Pre	50.503	9.921	52.604	12.246			0.018	0.895
	Tue Post	73.608	9.164	84.713	11.298			0.574	0.450
	Wed Pre	78.699	8.715	64.147	10.446			1.125	0.291
	Wed Post	80.432	10.446	65.888	11.312	0.277	0.600	0.877	0.351
	Thurs Pre	59.084	9.763	64.089	10.446			0.121	0.729
	Fri Post	79.035	9.193	84.123	12.297			0.108	0.742
	Sat Pre	31.212	9.205	23.340	12.335			0.257	0.613
	Sat Post	32.112	9.747	22.281	11.32			0.434	0.511
	Sun Pre	32.642	9.683	16.339	10.446			1.298	0.257

	Mean	56.847	3.162	54.062	3.853				
T_2 (pmol/L)	Baseline[a]	124.716	8.828	103.470	18.639				
	Mon Post	130.566	10.547	125.242	12.807			0.101	0.751
	Tue Pre	131.578	11.28	128.906	13.936			0.022	0.882
	Tue Post	109.118	10.435	131.993	12.864			1.873	0.173
	Wed Pre	127.487	9.908	128.870	11.875			0.008	0.929
	Wed Post	147.596	11.848	159.390	12.892	0.590	0.444	0.448	0.505
	Thurs Pre	143.047	11.055	155.414	11.875			0.575	0.450
	Fri Post	136.998	10.478	145.980	14.081			0.257	0.613
	Sat Pre	114.868	10.475	121.436	14.028			0.138	0.711
	Sat Post	131.784	11.068	127.028	12.826			0.078	0.781
	Sun Pre	81.299	11.006	76.647	11.875			0.082	0.775
	Mean	125.434	3.605	130.091	4.406				

EMM, estimated marginal means (accounting for baseline TH, weekly and daily ED); [a], EMM accounting for weekly and daily ED only; **bold and *** indicates significance at <.05 level.

7.3.4.1 Treatment and Time Effects

Analysis revealed a significant effect of treatment ($F_{(1, 134)} = 5.026$, $p = 0.027$) on T_4, with the intervention group (10.57 ± 0.46 pmol/L) having significantly lower mean T_4 concentration than the placebo group (12.38 ± 0.62 pmol/L) (Figure 7.6). Analysis also revealed a significant effect of time on T_4 ($F_{(9, 134)} = 2.196$, $p = 0.026$), with post-hoc comparisons identifying higher T_4 immediately post-match (14.06 ± 1.14 pmol/L) than immediately post-light training (Monday) (8.88 ± 1.14 pmol/l $p = 0.025$), when baseline variations were accounted for (Figure 7.7). No significant treatment*time interaction was identified around match or heavy training (Figure 7.8).

Analysis revealed a significant main effect of treatment on T_3 concentrations ($F_{(1, 133)} = 6.630$, $p = 0.011$) with significantly lower T_3 in the intervention group (15.11 ± 2.54) than the placebo group (21.78 ± 2.55) (Figure 7.6). Analysis also revealed a significant effect of time ($F_{(9, 132)} = 9.854$, $p = 0.000$) on T_3 concentration. Post-hoc comparisons identified significant higher T_3 pre-match (38.87 ± 4.35), post-match (36.526 ± 4.17) and 24 hr post-match (36.93 ± 4.37) than at all other time points when baseline variations were accounted for (Figure 7.7). A significant time*treatment interaction was identified ($F_{(9, 133)} = 2.067$, $p = 0.037$), post-hoc analysis identified significantly higher T_3 in the placebo group pre, post and 24 hr post-match (Figure 7.8).

Analysis revealed no significant main effects of treatment on rT3 concentration ($F_{(1, 145)}$ = 0.277, p = 0.600) (Figure 7.6), however a significant main effect of time was identified ($F_{(9, 145)}$ = 9.138, p = 0.000). Post-hoc analysis revealed significantly lower rT_3 pre-match (27.28 ± 7.63), post-match (27.15 ± 7.40) and 24 hr post-match (24.49 ± 7.09) than at all other time points when baseline variations were accounted for (Figure 7.7). No significant time*treatment interaction was found (Figure 7.8).

Analysis revealed no significant main effects of treatment on T_2 concentration ($F_{(1, 145)}$ = 0.59, p = 0.444) (Figure 7.6). However a significant main effect for time was identified ($F_{(9, 145)}$ = 6.35, p = 0.000), with post-hoc analysis revealing significantly lower T_2 24 hr post-match (78.97 ± 8.06) compared to all other time points (Figure 7.7). No significant time*treatment interaction was found (Figure 7.8).

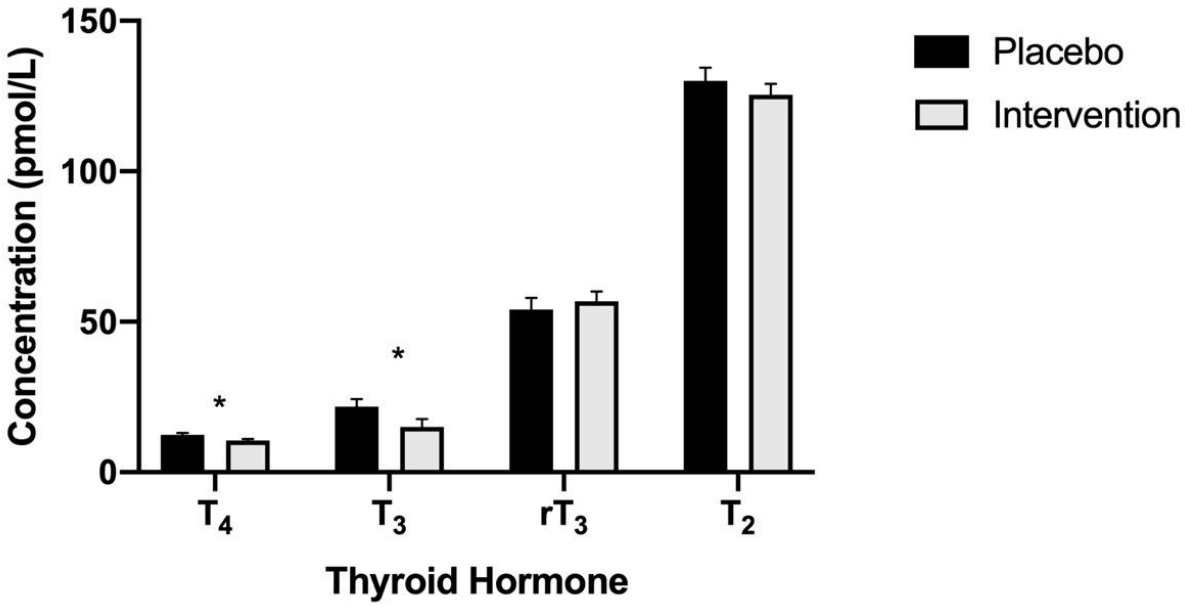

Figure 7.6 Difference in Mean Thyroid Hormone Concentrations Between Treatment Groups (EMM ± SD; *; significance at p < 0.05 level).

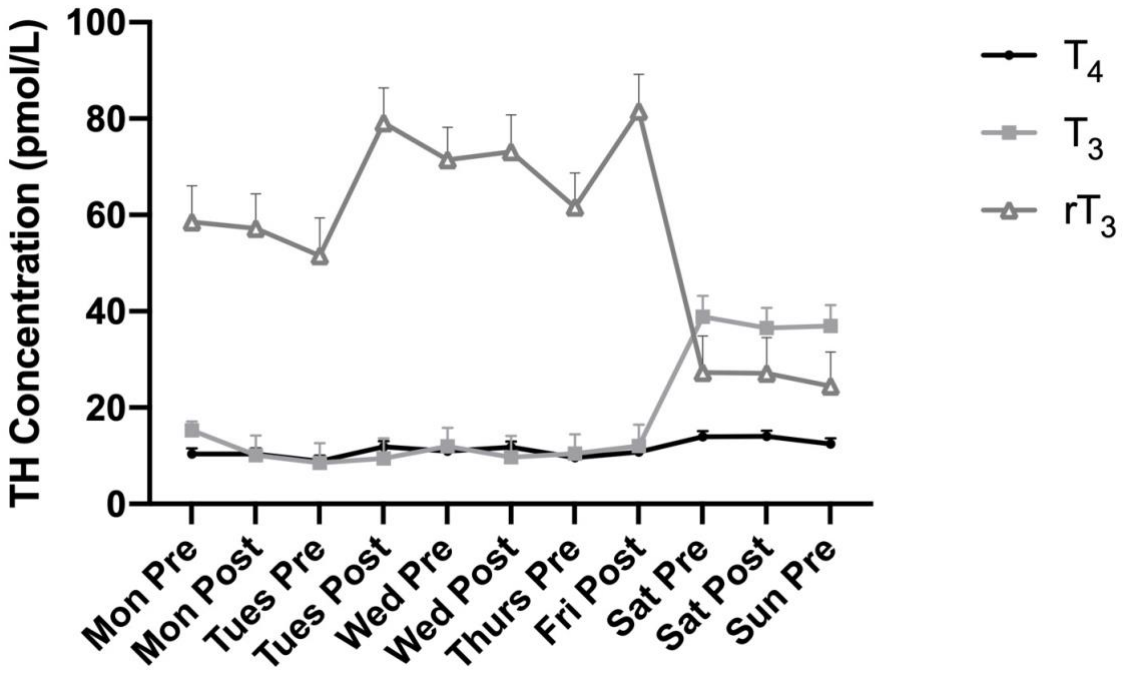

Figure 7.7 Changes in Mean Thyroid Hormone Concentrations Across A Competitive Football Week (EMM ± SEM).

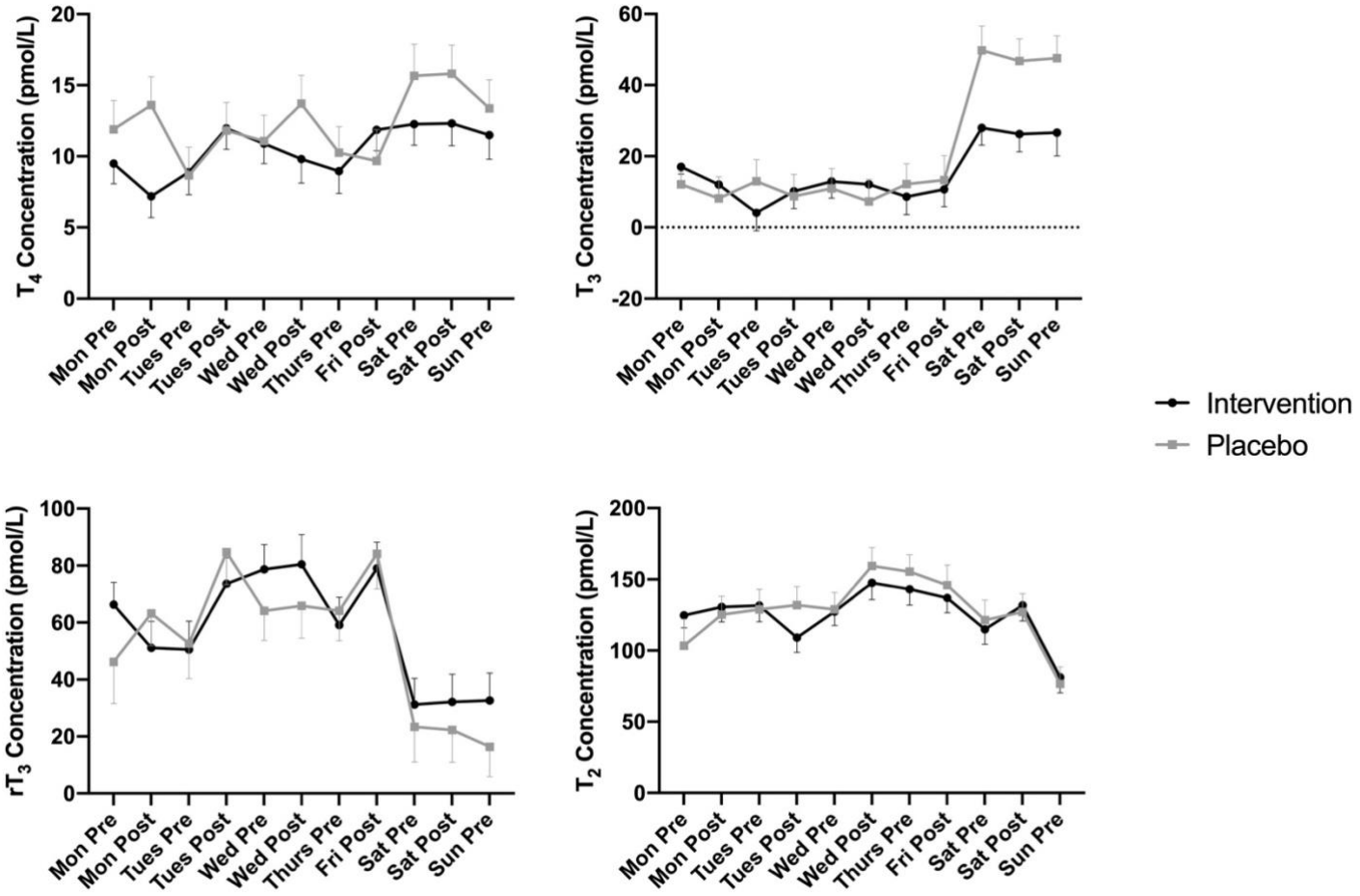

Figure 7.8 Changes in Thyroid Hormone Concentrations between Treatment Groups Across a Competitive Football Week (EMM ± SEM).

7.3.4.2 CO-FACTORS

7.3.4.2.1 Energy Deficit and Energy Availability

Energy deficit (mean, heavy-training day and match-day) was identified as a significant co-variate in all original models; post-hoc analysis therefore aimed to include these as main factors. However, due to literature supporting EA and there being established thresholds for EA it was decided to run post-hoc analysis on EA categorised according to established thresholds (Loucks et al., 2011) (Table 2.4).

Mean weekly EA categorised according to established thresholds (Loucks et al., 2011) (Table 2.4) did not have a significant effect on T_4, T_3, rT_3 or T_2 (Figure 7.9). When evaluating the impact of EA on match-days there was no significant differences between categories, however T_4 and T_3 concentrations were greatest and rT_3 lowest in the optimal category (Figure 7.10). Heavy-training day EA approached significance for T_3 ($F_{(3, 53}$ = 2.720, p = 0.054) with the optimal group having the greatest T_3, but was not significant for any TH. Although non-significant, results indicate alterations in TH concentrations between EA categories (Figure 7.11).

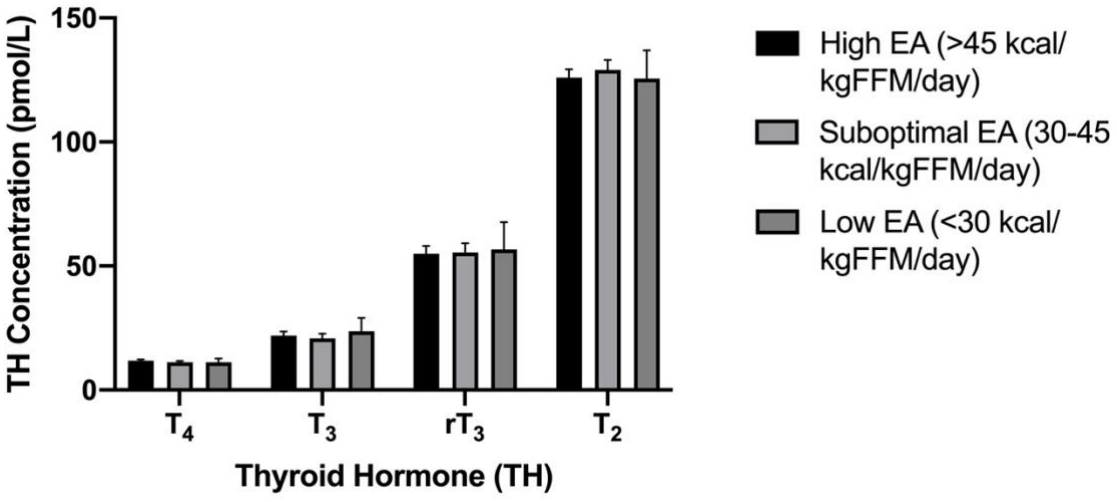

Figure 7.9 Mean Thyroid Hormone Concentration by Energy Availability (EMM ± SEM).

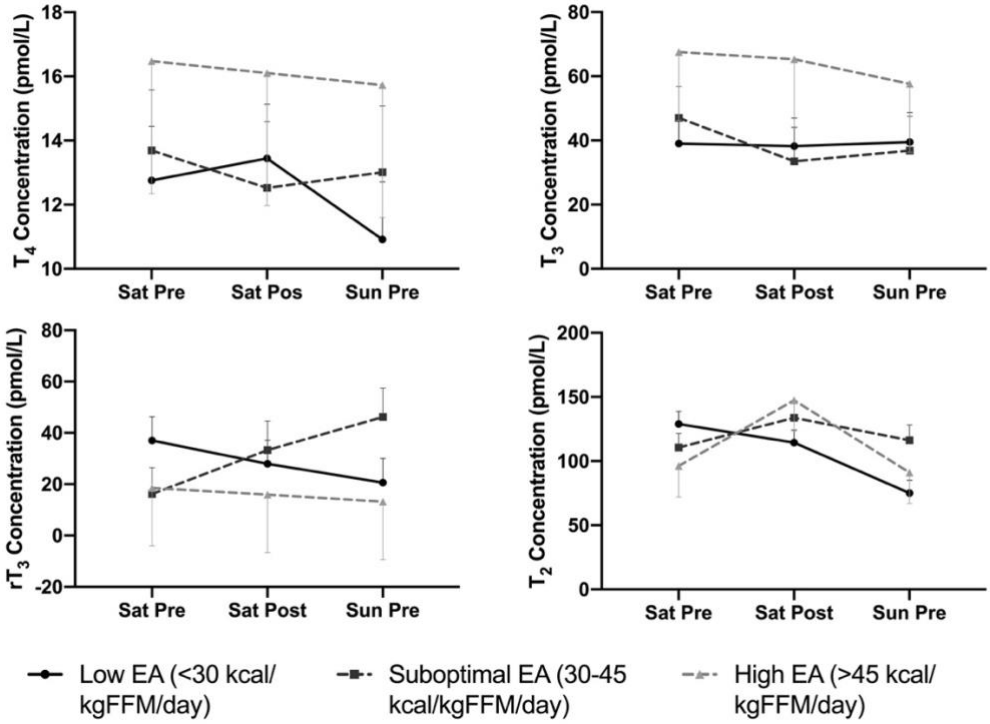

Figure 7.10 Change in Thyroid Hormone Concentration over the 24 hours Surrounding a Match Day Relative to Energy Availability (EMM ± SEM).

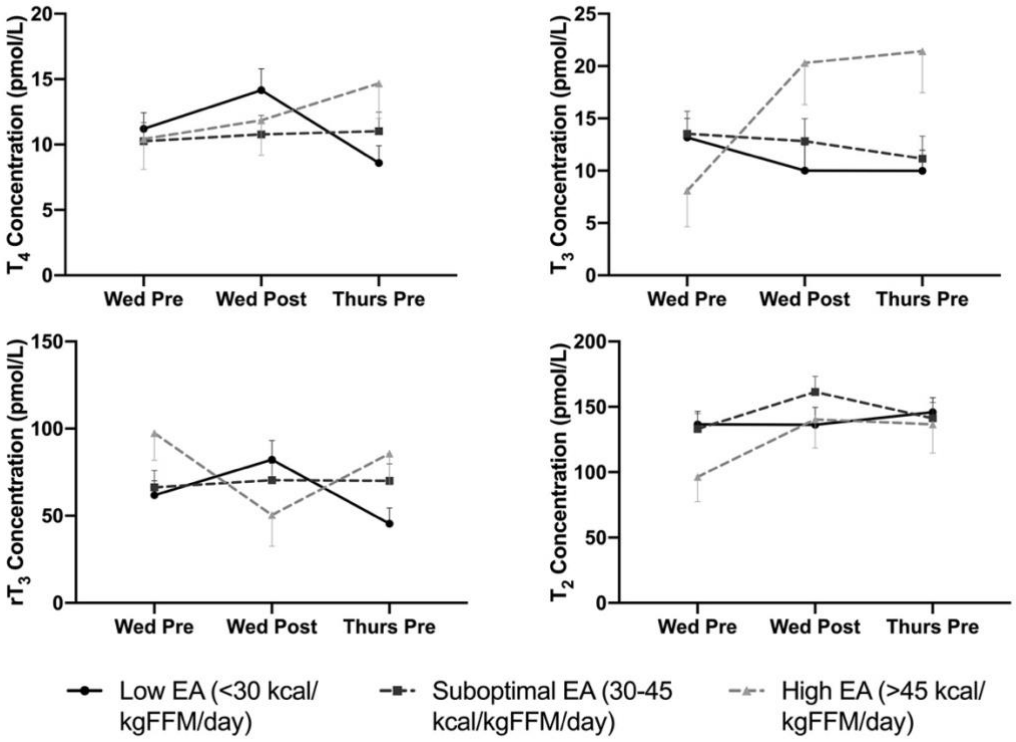

Figure 7.11 Change in Thyroid Hormone Concentration over the 24 hours Surrounding a Heavy Training Day, Relative to Energy Availability (EMM ± SEM).

7.3.4.2.2 Carbohydrate Intake

Carbohydrate intake (mean, heavy-training day and match-day) were identified as significant co-variates in all original models; post-hoc analysis therefore included these as main factors categorised as previously explained (Table 7.2).

Mean carbohydrate intake had a significant effect on T_4 ($F_{(3, 134)} = 4.398$, $p = 0.006$) and T_3 ($F_{(3, 98)} = 23.034$, $p = 0.000$). Post-hoc analysis identified significantly higher T_4 in group four than group two ($p = 0.024$) and a trend towards increased T_4 with increase carbohydrate intake; similarly, T_3 was significantly higher in group 4 than all other groups (Figure 7.12). Significant time*weekly carbohydrate intake interactions were identified for T_4 and T_3, post-hoc analysis identified significant differences largely surrounding MD and heavy training days (Table 7.6).

Table 7.6 Estimated Marginal Means and Effects of Time and Carbohydrate Intake on Thyroid Hormone Concentrations Across a Competitive Football Week

Hormone	Time	Mean Carbohydrate Intake Category (g/kg/day)				Main Effects			
		1	2	3	4	Mean Carbohydrate Intake		Mean Carbohydrate Intake*Time	
		< 3.198	**3.198 –3.804**	**3.804 –4.1004**	**> 4.1004**	***F***	***P***	***F***	***P***
T_4 (pmol/L)	Baseline[a]	11.47 ± 1.01	11.68 ± 0.74	10.56 ± 0.58	7.54 ± 0.32				
	Mon Post	**14.50 ± 1.90**	**7.28 ± 1.80**	**12.80 ± 1.80**	**5.64 ± 1.80**			**5.396**	**0.002**
	Tue Pre	4.50 ± 3.11	6.80 ± 1.74	9.55 ± 1.74	12.63 ± 2.20			2.147	0.097
	Tue Post	13.21 ± 1.90	11.44 ± 1.56	11.71 ± 1.56	10.44 ± 1.80			0.382	0.766
	Wed Pre	8.96 ± 1.90	8.57 ± 1.74	14.44 ± 1.70	14.40 ± 2.20			3.115	0.028
	Wed Post	11.12 ± 2.20	13.47 ± 1.80	12.39 ± 1.80	9.46 ± 2.20			0.732	0.534
	Thurs Pre	9.56 ± 1.90	7.23 ± 1.74	8.91 ± 1.74	16.70 ± 3.11	**4.398**	**0.006**	2.38	0.072
	Fri Post	10.84 ± 1.90	8.88 ± 2.20	11.43 ± 1.70	12.24 ± 2.20			0.437	0.727
	Sat Pre	**10.64 ± 1.90**	**17.97 ± 2.20**	**13.7 ± 1.70**	**17.64 ± 2.20**			**2.95**	**0.035**
	Sat Post	10.55 ± 1.90	16.68 ± 1.80	14.61 ± 1.70	16.97 ± 3.11			2.136	0.099
	Sun Pre	9.60 ± 1.90	14.92 ± 1.80	11.47 ± 1.80	16.11 ± 2.20			2.346	0.076
	Mean	*10.66 ± 0.63*	*10.44 ± 0.60*	*12.12 ± 0.56*	*13.22 ± 0.74*				
T_3 (pmol/L)	Baseline[a]	19.44 ± 0.64	11.34 ± 1.21	13.83 ± 1.06	19.03 ± 0.59				
	Mon Post	12.24 ± 3.40	17.29 ± 3.04	15.03 ± 2.98	14.29 ± 3.27			0.441	0.724
	Tue Pre	18.18 ± 5.15	11.46 ± 2.95	13.78 ± 2.89	12.18 ± 3.85			0.480	0.697
	Tue Post	15.01 ± 3.32	9.05 ± 2.72	19.00 ± 2.62	15.53 ± 3.27	**23.034**	**0.000**	2.628	0.053
	Wed Pre	13.77 ± 3.30	17.58 ± 2.94	11.63 ± 2.81	21.11 ± 3.82			1.712	0.168
	Wed Post	**21.92 ± 3.67**	**11.17 ± 3.05**	**11.52 ± 3.01**	**22.45 ± 3.95**			**3.638**	**0.015**
	Thurs Pre	7.52 ± 3.30	12.18 ± 3.01	9.34 ± 2.93	16.77 ± 5.42			0.957	0.415

	Fri Post	**21.05 ± 3.25**	**16.3 ± 3.81**	**7.38 ± 3.01**	**15.83 ± 3.88**			**3.484**	**0.018**
	Sat Pre	**39.13 ± 3.26**	**69.16 ± 3.85**	**42.11 ± 2.84**	**65.81 ± 3.94**			**21.252**	**0.000**
	Sat Post	**39.16 ± 3.26**	**66.95 ± 3.18**	**34.57 ± 2.87**	**73.02 ± 5.44**			**30.959**	**0.000**
	Sun Pre	**28.03 ± 3.25**	**66.41 ± 3.28**	**38.46 ± 3.03**	**63.77 ± 4.18**			**33.144**	**0.000**
	Mean	*21.78 ± 1.39*	*24.48 ± 1.29*	*20.35 ± 1.03*	*32.08 ± 1.89*				
	Baseline[a]	37.11 ± 6.66	65.50 ± 3.88	69.45 ± 2.22	57.46 ± 4.38				
	Mon Post	46.59 ± 11.50	43.8 ± 13.27	56.25 ± 13.27	77.51 ± 13.27			1.377	0.252
	Tue Pre	56.3 ± 22.99	33.61 ± 12.85	48.91 ± 12.85	40.97 ± 16.26			0.365	0.778
	Tue Post	**72.25 ± 11.50**	**91.83 ± 11.50**	**52.87 ± 11.50**	**104.12 ± 13.27**			**3.445**	**0.018**
	Wed Pre	83.85 ± 11.50	44.98 ± 12.85	74.38 ± 12.59	95.88 ± 16.26			2.545	0.058
rT_3	Wed Post	85.44 ± 14.08	64.30 ± 13.27	78.52 ± 13.27	73.71 ± 16.26			0.425	0.736
(pmol/L)	Thurs Pre	68.18 ± 11.50	39.25 ± 12.85	50.38 ± 12.85	14.86 ± 22.99	1.908	0.131	1.845	0.142
	Fri Post	75.94 ± 11.50	72.52 ± 16.26	100.32 ± 12.59	59.50 ± 16.26			1.509	0.215
	Sat Pre	23.2 ± 11.50	15.50 ± 16.26	33.24 ± 12.59	14.84 ± 16.26			0.375	0.771
	Sat Post	22.91 ± 11.50	15.57 ± 13.27	29.00 ± 12.59	20.00 ± 22.99			0.184	0.907
	Sun Pre	15.06 ± 11.50	11.34 ± 11.50	35.54 ± 12.85	13.47 ± 16.26			0.777	0.509
	Mean	*54.9 ± 3.92*	*43.91 ± 4.40*	*56.10 ± 4.10*	*51.49 ± 5.47*				
	Baseline[a]	96.25 ± 9.56	125.72 ± 3.41	140.32 ± 2.06	95.23 ± 1.20				
	Mon Post	154.27 ± 13.63	123.88 ± 15.74	135.51 ± 15.74	121.78 ± 15.74			1.067	0.365
T_2	Tue Pre	144.43 ± 27.26	117.02 ± 15.24	146.24 ± 15.24	172.88 ± 19.28			1.779	0.154
(pmol/L)	Tue Post	107.24 ± 13.63	130.45 ± 13.63	115.73 ± 13.63	118.15 ± 15.74	0.741	0.530	0.495	0.686
	Wed Pre	124.29 ± 13.63	140.06 ± 15.24	132.25 ± 14.93	114.09 ± 19.28			0.431	0.731
	Wed Post	**160.93 ± 16.69**	**110.11 ± 15.74**	**164.37 ± 15.74**	**177.01 ± 19.28**			**3.214**	**0.025**

Thurs Pre	148.6 ± 13.63	158.2 ± 15.24	152.17 ± 15.24	151.13 ± 27.26	0.075	0.973
Fri Post	140.87 ± 13.63	159.65 ± 19.28	160.84 ± 14.93	111.25 ± 19.28	1.620	0.187
Sat Pre	126.66 ± 13.63	122.26 ± 19.28	100.28 ± 14.93	116.32 ± 19.28	0.608	0.611
Sat Post	101.11 ± 13.63	138.28 ± 15.74	131.85 ± 14.93	134.16 ± 27.26	1.347	0.262
Sun Pre	77.49 ± 13.63	61.89 ± 13.63	87.37 ± 15.24	90.5 ± 19.28	0.728	0.537
Mean	*127.76 ± 4.65*	*127.4 ± 5.22*	*133.55 ± 4.86*	*130.73 ± 6.49*		

EMM, estimated marginal means (accounting for baseline TH, weekly and daily ED); ED, Energy Deficit; [a], EMM accounting for weekly and daily ED only; **bold** indicates significance at <.05 level.)

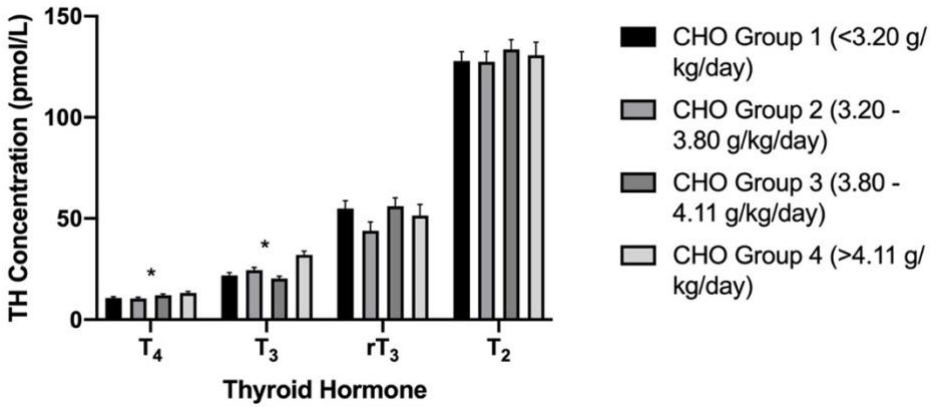

Figure 7.12 Mean Thyroid Hormone Concentration by Carbohydrate Intake (EMM ± SEM, *; significance at p < 0.05 level).

No significant main effects of match-day carbohydrate intake or match-day carbohydrate intake*time interactions were found (Figure 7.13). No significant main effect of heavy-training carbohydrate intake was found, however post-hoc carbohydrate intake*time interactions identified significant differences in T_4 pre-training as well as rT_3 and T_2 post and 24 hr post-training (Figure 7.14).

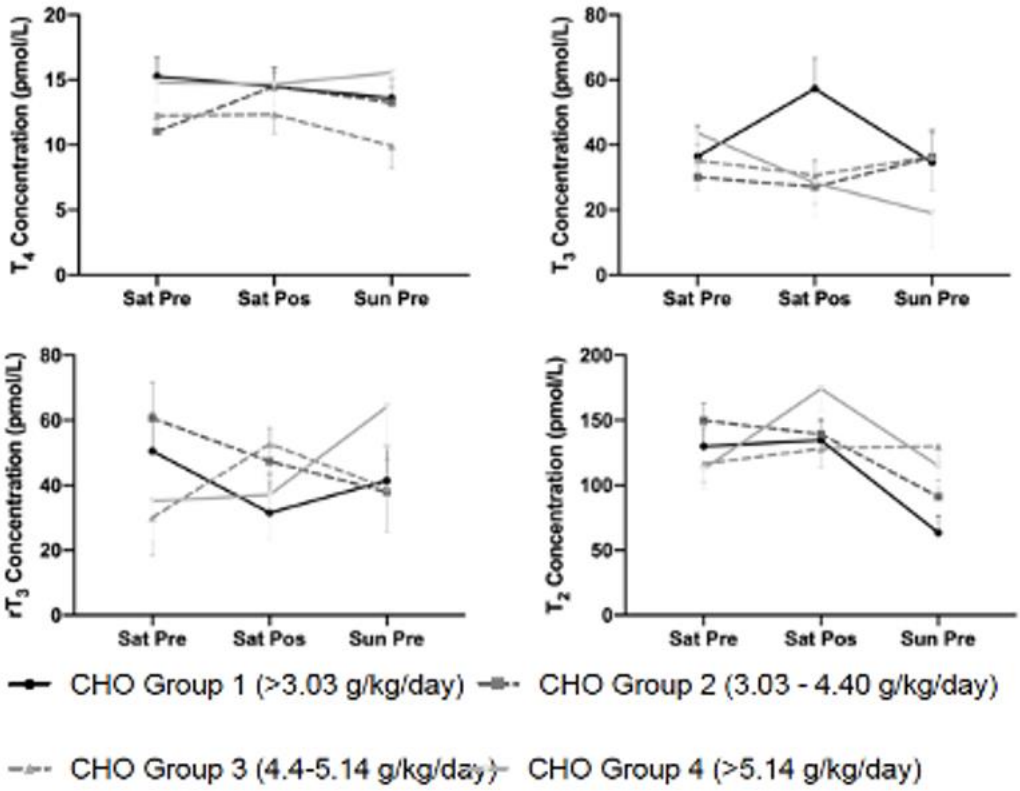

Figure 7.13 Change in Thyroid Hormone Concentration over the 24 hours Surrounding a Match Day Relative to Carbohydrate Intake (EMM ± SEM).

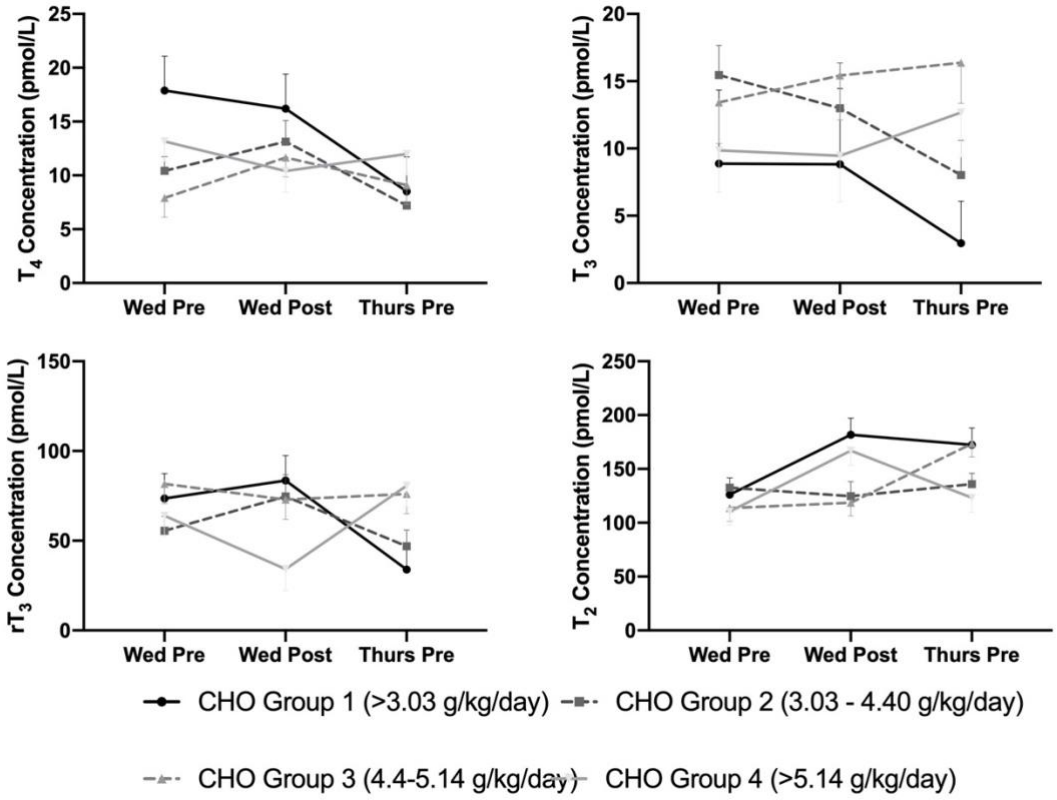

Figure 7.14 Change in Thyroid Hormone Concentration over the 24 hours Surrounding a Heavy Training Session Relative to Carbohydrate Intake (Mean ± SEM).

7.3.5 Impact of Intervention on BP and BT

No relationships were found between intervention or any covariates on BT or BP ($p > 0.05$ for all assessments).

7.4 Discussion

The present study aimed to investigate the acute impact of fructose and glucose co-ingestion following a competitive fixture and heavy training session on thyroid function in male adolescent football players. The data indicate that the ingestion of sucrose immediately following a competitive fixture attenuated an immediate decline in T_4 and T_3 and indicated that adaptations could last the following 24 hours, however this was not found to be significant, and the same interaction did not exist following a heavy training session. Moreover, when whole day free-living nutritional intake and EE were assessed both EB, EA and carbohydrate intake had a significant impact on T_4, T_3 and rT_3 with preferential TH concentrations identified with increased EA and carbohydrate intake.

Together the data suggest that a carbohydrate load post-match can attenuate a reduction in T_4 and T_3, however it is likely that these changes are related to the increased EA due to the intervention rather than the specific intervention utilised within this study. Furthermore, the data suggest that the co-ingestion of fructose and glucose was effective at attenuating the impact of a football match on THs in the short term (<60 minutes), however the longer-term impact appears to be mediated by overall EI, EA and carbohydrate intake. Together, these data present an integrative profile of the impact of competitive match-play, TL and nutritional intake on TH and supports the acute impact of match-day EI and carbohydrate intake on TH concentrations.

7.4.1 Carbohydrate Intervention on Thyroid Hormones

A carbohydrate load of 1.2 g/kg of sucrose consumed immediately following a competitive match attenuated the immediate post-match decline in T_4 and T_3. However, this preservation of T_3 was short-lived and at 24 hours post-match there was a shift towards rT_3 at the expense of T_3 whilst T_4 concentrations remained fairly consistent. These interactions indicate that although a carbohydrate load might acutely preserve thyroid function within the hour following the fixture, the duration of this impact is unknown and indicates towards a preferential peripheral conversion to rT_3 rather than T_3 24 hours post-match. The mechanisms behind this are unknown, however the direction and magnitude of alterations in T_3 reported within this study replicate those found 24 hours post exhaustive exercise (Hackney & Dobridge, 2009) and 12 hours after interval training (Hackney et al., 2012). Additionally, the data suggests that the carbohydrate intervention could mitigate the decline in T_3 post-exercise, but overall nutritional intakes, specifically EI and carbohydrate intakes, account for the longer-term implications of match play on thyroid functions. As such, increasing EI and overall carbohydrate intakes could have a more profound effect on recovery, subsequent athletic performance and health.

Although the intervention appeared to mitigate the impact of a fixture on thyroid function, the impact of the intervention around the heavy training day is less conclusive. The pattern of change for T_3 replicated that of MD, however this was not true for T_4 with the intervention group indicating a reduction in T_4 immediately post-training which

continued to decline in the following 24 hours. These combined changes 24-hour post-training could have notable effects on subsequent training sessions the following day (Nicoll et al., 2018). However, GPS metrics showed no significant differences in training between placebo and intervention the following day or alterations in morning wellness. Within the placebo group, rT_3 increased immediately post-training in conjunction with a slight decrease in T_3, supporting alterations in TH profiles for energy preservation in LEA as seen in other exercise studies (Hackney et al., 2012; Loucks & Callister, 1993). In contrast the intervention decreased rT_3 and increased T_3 immediately post exercise, indicating preservation of T_3 with a carbohydrate intervention. However, the changes in these hormones were marginal. As such, any impact of the intervention must be carefully considered and further research on a greater sample size is required to investigate this further. Although data from this study support the theory of altered TH profiles and conversion of TH in different EA states, and the protection of thyroid function with a sucrose load post-exercise, the reasoning for difference in responses between MD and heavy training are unclear. Alterations could exist due to the TL and type between match and training days, with greater HID, VHSR and A/D on match-days compared to heavy training days. This would support earlier findings of different TH interactions following interval vs steady state exercise (Ciloglu et al., 2005; Hackney et al., 2012; Kocahan & Dundar, 2018). Subsequently, variations in response to the intervention could be due to differences in fuel utilisation which were not evaluated within this study. However, alterations in carbohydrate metabolism could play a role in the intervention response following training and match play. Alternatively, the response could be related to the overall differences in EI and nutritional intake between days, with EI and carbohydrate intakes greater on heavy-training days compared to MDs.

Of note, is the significant variation in T_3 and rT_3 concentration between MD and heavy training day, irrespective of treatment group, with increased T_3 and suppressed rT_3 on MD. It could be hypothesised that this is related to the increased carbohydrate intake on MD compared to other days, which could negate the impact of LEA and increase or preserve T_3 (Kelly, 2000; Kose et al., 2017; Molteberg et al., 2020). However, the magnitude of increase in T_3 is greater than anticipated for the increase in EI and carbohydrate.

7.4.2 Energy Balance, Availability and Thyroid Hormones

In agreement with previous findings (Chapter 6) and existing literature (Heikura et al., 2017; Loucks et al., 1998; Loucks & Callister, 1993; Loucks & Heath, 1994; Loucks & Thuma, 2003) EB had a significant impact on baseline TH concentrations and the acute fluctuations in TH concentrations. Within this specific sample, EI values were similar to those within literature when assessed via the same method (Briggs et al., 2015a; Russell & Pennock, 2011) but lower than those more recently found using DLW (Morton, 2019).

The impact of mean ED on THs reached statistical significance for T_4, T_3 and T_2, with preferential thyroid profiles associated with a smaller ED. This supports findings from previous literature and the rationale that larger EDs result in increased alterations in peripheral TH conversion with preferential inner ring deiodination and increased rT_3 in an attempt to preserve energy (Heikura et al., 2017; Loucks et al., 1998; Loucks & Callister, 1993; Loucks & Heath, 1994; Loucks & Thuma, 2003) with potential detriments to athletic performance (Nicoll et al., 2018). Similarly, when considering energy as EA, data also supports the priority of adequate EA in support of thyroid function (Burke et al., 2018; Elliott-Sale et al., 2018; Loucks et al., 2011), with mean T_4 and T_3 values increasing with greater mean EA.

7.4.2.1 Match Day EA

When grouped according to established EA categories (Loucks et al., 2011), the data from MD EA and T_4, T_3 and rT_3 are largely expected, with those in optimal EA having the highest concentrations of T_4, T_3 and lowest rT_3 across the three time points, supporting the notion of increased thyroid function with increased EA (Burke et al., 2018; Elliott-Sale et al., 2018; Loucks et al., 2011). Although the optimal group had the most beneficial TH profile, the sub-optimal and LEA groups had relatively similar T_4 and T_3 profiles with differences mainly existing in rT_3 concentrations. The lowest EA category had a steady decline in rT_3 over the 24 hours period, whereas suboptimal steadily increased. The mechanism behind this is unknown, however this might be evidence to support a change in peripheral conversion to conserve energy at ~30 kcal/kgFFM/day, similar to the findings in untrained females of detrimental effects at ≤25 kcal/kgFFM/day (Hilton & Loucks, 2000; Loucks et al., 1998; Loucks & Heath, 1994). Alternatively, these

differences could be accounted for by other external factors. Due to the acute impact of the carbohydrate intervention on TH, it could be suggested that meal periodisation could play a role in these differences and impact on the rate of recovery of T_3 and rT_3. However, to date this has not been evaluated. In addition, composition of nutritional intake could impact on the findings, specifically with regard to carbohydrate intake within this study. Future studies could aim to establish the impact of post-match meal frequency, composition and timing on TH concentrations.

7.4.2.2 Heavy Training Day EA

Similar findings existed on heavy-training days, with the optimal EA category having the greatest mean T_3, with this increasing immediately post- and 24hr post-training in contrast to the decline seen in the other categories, with implications for recovery, subsequent training, athletic performance and injury incidences (Beyleroglu, 2011; Nicoll et al., 2018). However, in contrast to match-day TH concentrations, assessment of heavy-training day data also highlighted elevated T_4 and rT_3 in the LEA category pre- and post-heavy-training compared to the other categories, with a decline in both 24hr post-training. This pattern could suggest alterations in peripheral conversion of THs, away from rT_3, allowing T_3 to decline to a lesser magnitude than T_4, thus suggesting alterations in peripheral TH conversion not for energy preservation but in fact to alleviate the impact of LEA on T_3. Although these findings in part contradict those of the energy-conservation theory, they do support the priority of T_3 in the assessment and monitoring of thyroid function (Abdalla & Bianco, 2014). Furthermore, as this finding was only outlined on a heavy-training day, and the mean EA analysis did not reflect this, it could be understood that this is an acute preservation of T_3 in short periods of LEA. Although future research would be required to confirm this hypothesis, this could provide useful practical insight into THs during acute LEA periods which can exist during congested intense training such as in the pre-season or when athletes are attempting to reduce body mass.

7.4.3 Carbohydrate Intake and Thyroid Hormones

Existing literature supports the importance of adequate carbohydrate intake on TH (Kose et al., 2017; Molteberg et al., 2020), with indications that adequate carbohydrate intake can attenuate the consequences of LEA on thyroid function (Hackney & Dobridge, 2009;

Kelly, 2000; Reinhardt et al., 1993; Serog et al., 1982). Data from this study supports the importance of carbohydrate intake for thyroid function, with a trend towards increased mean T_4 and T_3 and accompanying decline in rT_3 seen with increased carbohydrate intake, the trend becoming significant on MD and heavy training days. Categories assigned to participants were based on quartiles, due to all participants being under the current carbohydrate recommendations (Burke et al., 2006; Desbrow et al., 2014). Although no significant differences were identified in heavy-training day TH concentrations, the lowest carbohydrate group had the highest T_4 concentration but lowest T_3, coinciding with increased rT_3 concentrations. This is similar to the data found in epileptic children and adults (Kose et al., 2017; Molteberg et al., 2020), indicating preferential conversion to rT_3 in low carbohydrate states. Although carbohydrate intake in this sample of adolescent male footballers was below guidelines even the category with the lowest intake was significantly greater than those used in the previous studies (~16 g/day). However, due to the increased training demands in adolescent male footballers compared to the sedentary participants in previous studies, the threshold in which carbohydrate intake supresses THs could be altered, specifically T_3. Due to the relatively similar carbohydrate intakes between all individuals (284 ± 97 g/day) it was not possible to determine in this study, however future studies could aim to outline categories of carbohydrate intake and assess when thyroid function is compromised, similar to the EA guidelines. In contrast, on match day the lowest carbohydrate intake group did not have differences in T_4 or rT_3 concentrations compared to other groups, and had the highest T_3 concentrations post-match, contradicting findings from heavy-training sessions. Differences between days could be due to the increased carbohydrate intake overall on match-day compared to heavy-training day (359±108 vs 284±97 g/day), further suggesting a threshold in which low carbohydrate intake impacts on TH concentrations. Alternatively, differences between days could be due to other external factors including individual TLs and modalities as previously discussed, or other stress factors which were not measured. Although there could be an indication that carbohydrate intake has an impact on TH concentrations, due to the sample size and free-living conditions not tightly controlling EI and carbohydrate intake within this study, it was not possible to evaluate if those changes occur irrespective of EI. As such, future studies could aim to assess if adequate carbohydrate intake could mediate the impact of LEA on thyroid function and, as such, utilise these to develop interventions and nutritional recommendations to preserve thyroid

function in periods of LEA, such as during congested fixtures and in weight-making sports.

7.4.4 Body Temperature and Blood Pressure

Unlike the findings from the controlled validity and reproducibility studies (Chapter 4 and 5) there were no clear relationship between BT, SBP, DBP and any THs. This is thought to be due to the large variation in outdoor temperatures over the period of training and the impact of exercise on BT and BP which could have compounded the results despite providing a rest period prior to collecting data. Future studies should aim to establish if morning wake time BT and BP correlate with TH concentrations and determine if this could be used within applied sport settings as a means to tracking thyroid function and metabolic rate changes.

7.4.5 Methodological Considerations

Although this study allowed free-living assessment of the impact of a carbohydrate intervention on TH concentrations, and allows for rapid application into practice, the study design has inherent limitations. Firstly, the sample size available was constrained to full-time scholars within a Category One academy which became further limited due to player unavailability due to injury, selection for national squads or older age groups. In addition, despite the methods of dietary intake being valid within this population (Briggs et al., 2015b), it relies on accurate and honest responses from athletes which cannot be guaranteed. Future studies might choose to build on recent DLW studies in this population (Morton, 2019) to assess the impact of EA on thyroid function using gold standard methods. Furthermore, there were no opportunities to gather RMR data on these athletes due to time constraints and player availability, as such although it is useful to collect TH data it would be beneficial to correlate this with adaptations in RMR and assess the acute impacts as this has not been examined. Furthermore, data from this study has outlined that although a trend does exist for ED, EA and carbohydrate intake on THs there might be particular points in which THs become most compromised. As such it would be beneficial to design a more controlled trial to assess the impact of varying EA and carbohydrate intake on THs to outline where thyroid function might become at risk. Furthermore, due to the congested fixture periods players are often exposed to, it would

be beneficial to extend the methods used in this study to assess the impact of multiple matches on thyroid function and if a carbohydrate intervention can continue to improve thyroid function following a competitive fixture. Finally, although there are indications that co-ingestion of glucose and fructose post-match can attenuate the impact of match play on THs, it would be beneficial to assess if other carbohydrate types or quantities have a similar or more profound impact on THs.

7.5 Conclusion

This study demonstrated that the co-ingestion of fructose and glucose immediately after a heavy training session and competitive fixture has acute benefits to TH concentrations lasting less than 60 minutes. However, to negate the consequences of increased training and match demands on thyroid function, more prolonged nutritional alterations need to be considered, including EA and carbohydrate intake. Data from this study suggests that in adolescent male athletes EA <30 kcal/kgFFM/day results in alterations in peripheral conversion of TH with increased rT_3 concentrations. Furthermore, data suggest there might be a point at which low carbohydrate intake dictates TH conversion, however it was beyond the scope of this study to define this threshold. Collectively, the data support the importance of EA and carbohydrate intake for thyroid function, and that adjustments in both of these factors can impact on THs. Data also suggest that a post-training sucrose load can acutely attenuate the negative alterations in TH concentrations which could be incorporated into more systemic nutritional interventions.

Chapter 8:
General Discussion and Conclusions

8.1 Experimental Recap

The overall aim of this thesis was to investigate the impact of maturation, TL and nutritional intake on thyroid function in elite adolescent male footballers. To achieve this Chapter 4 and 5 aimed to establish practical and reliable methods of assessment of thyroid function within this population. These included alternative methods for quantification of THs requiring smaller blood samples obtained through finger-tip capillary sampling, in addition, alternative non-invasive surrogate markers were assessed for reliability and relationships to TH concentrations. Chapter 6 subsequently implemented these methods to establish the free-living impact of maturation, TL and nutritional intake on thyroid function in adolescent footballers. Finally, using the findings from Chapter 6, periods were identified in which thyroid function might be compromised. Subsequently Chapter 7 aimed to preserve thyroid function at these times through an acute carbohydrate intervention. This chapter will collectively review and discuss the main findings of this thesis within the context of existing literature. In addition, insights will be provided into the practical applications of such findings and directions for future work will be suggested.

8.2 Summary of Main Findings

In Chapter 4 it was shown that digital alternatives of BT and BP assessment possess modest to strong criterion validity compared to gold-standard assessment methods. Notably, there was systematic under-prediction in the digital thermometers and as such a predictive equation was established for use in future studies. Chapter 5 extended these findings and outlined that the methods were reliable within adolescent males if test conditions are standardised. Chapter 5 also established LC-MS analysis of TH concentrations as a valid and reliable alternative to ELISA methods, outlining the merits of this method particularly in the applied setting. Furthermore, Chapter 5 outlined the TE scores for all TH across a 24-hour period which were subsequently utilised in future experimental studies to interpret findings and inform study design, with sample size estimations based on these findings. Finally, Chapter 5 established a link between BT, T_4 and T_3 indicating that this could be implemented as a practical surrogate marker of thyroid function if test conditions are standardised, as outlined in Chapter 4. Although Chapter 5 outlined a link in BP and T_3 the data were less conclusive. The decision was made to

continue to assess BP due to the ease of data collection and no extra strain on the participant, however subsequent data were scrutinised closely. Chapters4 and 5 outlined that LC-MS is a valid alternative for quantifying circulating TH concentrations and identified that this is a practical and preferable method to be used in adolescent populations. Furthermore, these chapters indicated that BT assessment could be utilised as a surrogate non-invasive marker of thyroid function. Thereafter, subsequent studies in this thesis used LC-MS analysis to quantify circulating TH concentrations and continued to monitor BT and BP as surrogate markers of thyroid function.

Chapter 6 outlined the potential risk factors for supressed thyroid function in adolescent athletes, highlighting that maturation, EA and carbohydrate intakes are determinants of TH concentrations within this population. Accordingly, this chapter highlighted that those in circa PHV, in suboptimal or low EA (<45 kcal/kgFFM/day) and with low carbohydrate intakes (<3 g/kg/day) were more likely to have supressed T_3. Furthermore, this chapter highlighted that there was greater alteration in TH concentrations in the 24 hours following a competitive fixture, with supressed T_4 and T_3 and elevated rT_3. Together, the findings from this study outlined the importance of maintaining adequate EA, particularly following high TL such as during a match, and that increasing carbohydrate intake could potentially mitigate some of the consequences of suboptimal EA on T_3.

Chapter 7 was designed to build on the findings from Chapter 6 and the methods developed in Chapter 4 and 5 to explore the acute impact of a carbohydrate load following the most physically demanding sessions of the week, a MD and heavy-training day. Although Chapter 6 identified that those in PHV were at risk of supressed thyroid function, it was not feasible to conduct this study on individuals in this maturation stage and as such, this study attempted to negate the impact of LEA and increasing TL on thyroid function. Co-ingestion of fructose and glucose attenuated the decline of T_4 and T_3 immediately post-match, however, this finding did not remain 24 hours later, indicating that any benefit of the carbohydrate load is short-lived. Additionally, this study indicated that LEA can supress T_3, as expected and supported to the findings from Chapter 6. Furthermore, this study suggested that longer-term alterations and preservation of thyroid function during periods of stress is more likely to be obtained through modulating

adequate EI and optimal EA. Collectively, the results presented in this thesis add to the current body of literature by suggesting maturation, TL and EA impact on thyroid function, and suggest that more systemic nutritional interventions are required to modulate the decline in T_3 seen during times of stress. The explanations for the aforementioned findings are discussed in greater detail below with considerations for the methods utilised, future research and practical implications of repeated periods of low TH status presented.

8.2.1 Methods of Assessing Thyroid Function

One aim of the present thesis was to determine the most appropriate methods for assessing thyroid function in adolescent athletes within the applied environment. Although this was predominantly achieved through the validity and reproducibility studies in Chapters 4 and 5, data and experimental experience provided in subsequent experimental chapters provided additional insight into the practical implications and limitations of these methods.

The use of LC-MS in the quantification of TH concentrations was deemed as reproducible in Chapter 5 and subsequently successfully implemented into other experimental trials. A clear benefit of LC-MS analysis over ELISA methods is the smaller blood samples required (~20 μL) which can be obtained by finger-tip capillary sampling which is less invasive and simpler than antecubital-venous sampling (Nunes et al., 2006; Rondeel et al., 2010) and had good compliance within these studies with only one participant across three studies withdrawing due to problems with the blood sampling. Furthermore, the LC-MS analysis allows reliable quantification of rT_3 which is not achievable from current ELISA methods (Bowerbank et al., 2019; Li et al., 2014; Masika et al., 2016). As initially hypothesised, rT_3 can provide valuable insight into the origins of thyroid function problems, specifically where issues are believed to be due to stress factors impacting on peripheral conversion (Abdalla & Bianco, 2014; Li et al., 2014; Masika et al., 2016; Thienpont et al., 2013b; Wang & Stapleton, 2010). Findings from Chapter 6 and 7 further support this and its importance with significant variations in rT_3 concentrations depending on training day, EA and maturation status. However, despite there being no systematic change in any TH between or within days for all THs, Chapter 5 highlighted the larger

random error in the measurement of rT_3 and T_2 at 80 minutes (TE = 13.75 and 12.45 pmol/L respectively) and 24 hours (TE = 18.77 and 21.96 pmol/L respectively) than T_4 and T_3 which must be considered when interpreting the findings. Outcomes from Chapter 5 also indicated a large individual variation in TH concentrations and therefore supports the development of narrower reference ranges (Andersen et al., 2002; Hollowell et al., 2002; Thienpont et al., 2013b; Wartofsky & Dickey, 2005) and also had direct implications for analysis of subsequent studies, which accounted for baseline TH concentrations through incorporation into all models. This should also be considered when assessing any TH concentrations, particularly within the exercise setting when fasting, EA and exercise can impact on THs. As such, it could be advised to take baseline TH concentrations in standardised conditions if planning to monitor TH concentrations, furthermore due to the documented circadian fluctuations in THs (Russell et al., 2008) further evidenced in Chapter 5 with greater within-day variation in T_4 and T_3 than between-day, monitoring should be taken at a consistent time away from PA bouts.

Surrogate markers of thyroid function would allow for non-invasive monitoring of thyroid function with clear benefits in the applied setting. Both BT and BP have been suggested as potential surrogates due to their known relationships with TH concentrations (Asvold et al., 2007; Chen et al., 2012; Fommei & Iervasi, 2002; Ittermann et al., 2012; Luke et al., 2004; Saito et al., 1983). Chapter 5 aimed to establish if these methods are appropriate in monitoring thyroid function in adolescent athletes. Initial findings from Chapter 4 and 5 suggested that digital methods of assessing BT and BP had modest to high validity and reproducibility in adolescent males during controlled trials at rest. Subsequently, both BT and BP were found to respectively track and inversely track T_3 concentration in rested conditions, supporting the notion of these being indicative of thyroid status. However, when implemented into practice in the exercise setting in Chapter 6 and 7, these methods appeared insensitive to acute variations seen in TH concentrations. It is hypothesised that this is due to the transient impact of exercise on BP and BT, with an increase in BT and BP post-exercise.

Therefore, although data from the controlled reproducibility study (Chapter 5) indicated BT and BP monitoring could provide non-invasive insight into thyroid function, this method was not appropriate for post-exercise monitoring. Accordingly, future studies

might aim to investigate the association of daily controlled assessment of BT and TH concentrations. If successful, then it is feasible that daily BT monitoring, similar to that of daily wellness monitoring which is commonplace within elite environments (Coyne et al., 2018), could act as an affordable, non-invasive surrogate for monitoring thyroid function and establishing when further investigation is required, potentially through LC-MS analysis.

8.2.2 Impact of Energy Availability on Thyroid Function

Chapter 6 and 7 demonstrated a change in TH concentrations in different EA states, with findings from both studies indicating a progressive decline in T_4 and T_3 with decreasing EA, in accordance with existing literature (Heikura et al., 2017; Loucks et al., 1998; Loucks & Callister, 1993; Loucks & Heath, 1994; Loucks & Thuma, 2003). However, the data for rT_3 is less conclusive, with Chapter 7 indicating a slight increase in LEA supporting the energy preservation theory (Boelen et al., 2008) whilst in Chapter 6 rT_3 increased with greater EA alongside T_4 and T_3. The results from Chapter 6 are suggestive of a more allostatic adaptation to the HPT axis in response to LEA (Chatzitomaris et al., 2017), resulting in suppression of all TH and less supportive of alterations in peripheral conversion of THs. Furthermore, the reduction in T_4 is greater than those previously outlined (Loucks & Heath, 1994) and reported in Chapter 7, potentially due to the individual's maturation (Rubenstein et al., 1973) or training status (Hawamdeh et al., 2012). In contrast findings from Chapter 7 are more consistent with current literature and the suggestion that alterations in TH production in LEA is due to a shift in peripheral conversion away from D1 to D3 to preserve energy (Boelen et al., 2008). Although no data reached statistical significance for the mean TH concentrations, the results do indicate that, in free-living conditions, differences in EA do occur and there are the proposed consequences to thyroid function.

One of the aims of the thesis was to outline thresholds at which thyroid function could be at risk, within this thesis the existing EA criteria (Loucks et al., 2011) was used in an attempt to do this, and did allow greater visualisation and interpretation of the EA findings. Data from the studies suggested that EA below ~30 g/kgFFM/day had implications for TH concentrations, however due to a lack of individuals falling within

the ‘optimal’ category across both studies, and lack of players classified as ‘high’ on a MD, it is difficult to confirm these indications. As such, although these findings do support the suppression of T_4 and T_3 with LEA and the use of established EA thresholds, further research is required across a range of EA to confirm if there is a threshold at which thyroid function becomes at risk. In addition, the findings from this study and differences in findings between Chapter 6 and 7, particularly with regard to alterations in rT_3, could suggest the need for these to be investigated in different maturation statuses as the combined impact of EA and maturation could differ.

Combined data from both studies indicated significant differences in EI and EE on heavy training days and MDs, resulting in lower mean EA on these days than compared to rest and light training days. This supports previous research (Briggs et al., 2015a; Russell & Pennock, 2011). The findings from both these studies outline that LEA on these days also had a greater impact on TH concentrations, reaching statistical significance on heavy-training days, and that this effect remained up to 24 hours after the training bout. Within the sporting environment this decline has clear implications for health but also training performance, the remaining detriments 24 hours post-exercise also has clear connotations for recovery and subsequent athletic performance. The more apparent detrimental impact on these days could be down to the lower EA reported as previously outlined but could also be compounded by other factors. Both match and heavy-training days report greater overall TL with known impacts, in addition both days report the largest HID within Chapter 7 which aligns with current knowledge that HIIT reduces T_4 and T_3 more than steady-state exercise (Hackney et al., 2012). Of particular note is that the impact was greater on heavy-training days compared to MDs in both Chapter 6 and Chapter 7. This was unexpected due to the lower EA and higher TL reported on a MD. The mechanism for this is unknown, however it could be due to other significant factors as previously explained, particularly carbohydrate intake which is greater on MD-1 and MD than in the days surrounding the heavy training session, thus supporting the finding that under LEA an increase in carbohydrate intake might attenuate negative consequences, albeit not entirely. Although in Chapter 7 EA was manipulated through the use of a carbohydrate load post-exercise, this was not great enough to exert a significant effect on EA and attenuate the negative consequences of LEA on thyroid function. Accordingly, it might

be more appropriate to increase overall EA to a particular level for the carbohydrate intervention to exert a significant impact.

Of notable practical importance is the lack of optimal EA reported on the most demanding training days across both Chapter 6 and Chapter 7 which must be addressed. Although not a primary aim of the thesis, the finding that despite previous research outlining significant ED within this population a significant LEA still remains needs attention. As such, different nutritional provision approaches might need to be taken. Furthermore, the evidence that this exists across u12-u18 level indicates that this support might need to be implemented at younger age groups to instil positive nutritional intakes to support EA.

8.2.3 Nutrient Intakes and Thyroid Function

Beyond the impact of EA on TH concentrations, the data from Chapter 6 and 7 suggest that the intakes of other nutrients influence thyroid function. The impact of selenium, iron and iodine intakes on thyroid function are already established (Larson-Meyer & Gostas, 2020; Ventura et al., 2017). However, the data from this thesis suggest that although the majority of micronutrient intakes were within reference values, potassium, calcium, selenium and iodine were below RNI values. However, within these studies there was no significant impact of any micronutrient on thyroid function. Accordingly, within this specific sample there is no immediate concern for the impact of micronutrient intake on thyroid function, potentially due to bigger factors such as EA and maturation. However, future studies might look at investigating the micronutrient intakes in adolescent athletes in optimal EA and establish if there are any issues. In contrast, this thesis outlined that carbohydrate intake has a significant impact on TH concentrations, specifically T_4 and T_3. Findings outlined that T_4 and T_3 were significantly lower in lower carbohydrate intake groups, supporting findings from non-athletic populations (O'Brian et al., 1980; Reinhardt et al., 1993; Serog et al., 1982). Although previous literature has predominantly investigated the impact of very low carbohydrate intakes, including ketogenic diets, on thyroid function, results from Chapter 7 suggested that significant differences in thyroid function exist when carbohydrate intake is below ~ 4 g/kg/day. Although data from this study suggests that a threshold might exist for supressed THs with low carbohydrate intake, more data are needed to quantify this. Furthermore, this threshold is proposed to

be higher than those previously suggested in sedentary or fasted populations (Kelly, 2000), however it is hypothesised that this is due to increased carbohydrate metabolism and requirements within footballers.

In adolescent male footballers, it is difficult to fully ascertain if the decline in T_3 is due solely to low carbohydrate intakes or is in part a further consequence of the LEA that often occurs due to low carbohydrate intakes. However, findings from habitual dietary intakes and from the acute carbohydrate intervention do suggest that increased carbohydrate intakes can attenuate the consequences of LEA on thyroid function, as previously suggested in ketogenic studies (Kose et al., 2017; Molteberg et al., 2020). Therefore, the data from this study support the prioritisation of optimal EA and carbohydrate intakes in support of preserving thyroid function, particularly during periods of increased stress and EE. It can be postulated that the dietary intake of carbohydrates required to offset the consequences of LEA on thyroid function is higher in athletic populations due to the increased carbohydrate oxidation and subsequent requirements (Eriksson & Saltin, 1974; Timmons et al., 2003, 2007). This is of particular importance due to the low carbohydrate intakes reported in Chapter 6 and 7, and in previous literature (Briggs et al., 2015a; Naughton et al., 2016; Russell & Pennock, 2011). As such, the findings from this thesis combined with existing literature, support the prioritisation of optimal EA and carbohydrate intakes within adolescent footballers, in support of preserving thyroid function, particularly during periods of increased stress and EE.

8.2.4 Impact of Maturation on Thyroid Function

Chapter 6 outlined maturation status as a potential risk factor for supressed thyroid function in adolescent male footballers. Players in circa-PHV had lower T_4 and T_3 concentrations than post-PHV, however no individuals were classified as pre-PHV. Although T_3 and RMR are believed to decline almost linearly with age, there is also an indication of an abrupt decline in T_3 around the onset of pubertal growth (Rubenstein et al., 1973). The aforementioned research, however, did not collect maturation status information and findings were based solely on chronological age with clear limitations. By assessing maturation, albeit through non-invasive measures, the findings highlighted within this thesis outline the suppression of thyroid function during PHV. In addition, and

in contrast to current understanding, the findings from Chapter 6 outlined maturation significantly impacting on T_4, with those in PHV having significantly lower T_4 than T_3. This contradicts the initial hypothesis of alterations in TH concentrations being due solely to peripheral conversion of T_4 and suggests allostatic adaptations to the HPT axis (Chatzitomaris et al., 2017). Furthermore, data from Chapter 6 and 7 suggested that concentrations of all TH were lower in the u14 – u16 age categories than compared to the u18s, despite 42% of the u14 – u16s being classified as PHV. Accordingly, there are two possible mechanisms for this finding. Firstly, it could imply that there are other factors not assessed within this study impairing thyroid function, such as psychological stress (Fommei & Iervasi, 2002). As such, future studies could aim to quantify psychological stress and determine other factors or investigate the relationship between cortisol and THs as a main outcome. However, as cortisol is known to impair the function of D1 and allows D3 to be more active peripherally (Hackney et al., 2012), it would be postulated that additional stress factors would impair T_3 and increase rT_3 but not impact on T_4 concentrations as identified within this thesis. Secondly, this finding could imply that categorising individuals using PHV as a measure of maturation is not accurate enough to fully outline the impact of maturation on TH concentrations. Due to the large variation within each category (Table 3.1), this could lack the specificity to outline the true impact of maturation and highlight if there is a specific maturity offset or biological age at which thyroid function is most at risk. As such, assessment of skeletal age could provide more accurate insight into maturation status and allow more thorough assessment of the impact of maturation status on TH concentrations.

Findings from this thesis highlight the impact of maturation on thyroid function and together provide novel information regarding the need for specific TH concentration reference values and monitoring of THs particularly amongst those in circa-PHV. Furthermore, the results indicate the importance of maintaining optimal EA and carbohydrate intake, particularly with PHV, to mitigate any consequences of simultaneous LEA, maturation and low carbohydrate intake on thyroid function.

8.3 Practical Application

Based on the findings from the studies in this thesis, some practical recommendations can be drawn for the assessment and preservation of thyroid function in adolescent athletes

and important factors for maintaining thyroid function, namely EA and carbohydrate intake. Firstly, there is a large inter-individual variation in baseline TH status within academy soccer players including those of similar maturation, body composition and EA status. Similarly, the magnitude of change in THs following a bout of stress has large individual variation. Combined, these highlight the importance of assessing baseline TH status and monitoring thyroid function across the season. Furthermore, this could suggest a need and benefit of athlete reference values for TH concentrations. Similarly, the initial data from this thesis suggests that daily BT monitoring could offer an affordable practical surrogate for thyroid function monitoring, however a note of caution is due here since these findings were not appropriate for monitoring acute changes in THs following exercise.

Secondly, this thesis adds to the previous literature outlining suboptimal nutritional intakes within adolescent footballers (Briggs et al., 2015a; Morton, 2019; Naughton et al., 2017; Naughton et al., 2016; Russell & Pennock, 2011) as EI and carbohydrate within this population did not meet the recommendations. Of greater importance is the finding that some EI do not support adequate thyroid function, suggesting incidences of RED-S in adolescent male footballers. Accordingly, this has practical implications for the provision and priority of nutrition support within academy environments. Finally, data from this thesis outline that assessment of THs is possible through LC-MS analysis and through finger-tip capillary blood sampling which is tolerable within adolescents. Importantly, this research highlights that this can successfully be implemented into the applied setting to assess and monitor THs. Although this might not be possible across the full season this could be conducted when thyroid function might be at risk including during maturation and following intense periods of training or fixtures, including pre-season.

8.4 Limitations

A number of limitations exist within this thesis, those related specifically to methods used within individual studies are addressed in respective chapters (Chapters 4-7). However, the following section discusses more general limitations associated with the thesis as a whole, which should be considered within the context of the elite sporting environment

in which the research was conducted. Paramount to this are the small sample sizes, limited opportunities to manipulate the training programme and frequent last-minute adaptations in schedules impacting on research.

Firstly, the key findings from the studies involving academy footballers are conducted involving a relatively small group of individuals from one category one football academy in England. Due to the different training philosophies, amount of nutritional support, background of players and the physical status of players these data are therefore not representative of all academy or elite level football players. As such, to generalise to a wider population more data are needed. Furthermore, the sample sizes for each experimental chapter are relatively small and have relatively high dropout rates or missed time-points due mostly to injuries and the movement between squads. Although a LMM approach was used to account for missed timepoints and dropouts, the small sample sizes mean the results require careful interpretation. In addition, due to the demanding schedules academy players undergo alongside their school and home life it was not feasible to conduct the final experimental study (Chapter 7) in individuals undergoing maturation, which had previously been identified to impact on thyroid function (Chapter 6). Although the data and findings from Chapter 7 provide novel insight into the impact of a carbohydrate intervention and EA on thyroid function, the benefits of this might be more pertinent in a group undergoing PHV. The techniques used within this research allowed an affordable assessment of nutritional intake, EE, BT and BP however these all require data from prediction equations, rely on participant compliance and might be objects of under-reporting bias. Although all methods have been validated, they are not the gold standard and as such have inherent limitations. Future work might aim to build on this research and recent studies utilising DLW within this population for accurate EB assessment.

In addition, due to the nature of the study all data were collected in males and therefore cannot be generalised or extrapolated to females, particularly due to the reported sex differences on TH concentrations. As such it would be advantageous to extend on this body of work by investigating elite female adolescent athletes. The data from this study is gathered from a series of acute studies and as such, the longer-term impact of EA,

maturation and training load on THs is unknown. It would, therefore, be advantageous to evaluate the variation in THs across a whole football season and the impact this has on health and athletic performance. As mentioned in Chapter 6 and 7 there were no opportunities to assess RMR in these athletes which would be advantageous when assessing TH concentrations. In summary, it would be beneficial to assess the impact of TH on RMR and other performance markers across a football season.

8.5 Future Directions

Building upon the findings from this thesis as well as time spent working within the field, further research is required to advance our knowledge of thyroid function in adolescent athletes and ultimately how we can better support the players development, athletic performance and health.

Whilst this research has focused on the acute thyroid responses to multiple variables, several questions remain unanswered including the longer-term impact of these demands on adolescent footballers. Data suggests variation in THs between athletes and non-athletes (Hawamdeh et al., 2012; Nicoll et al., 2018). Chapter 7 supports this with T_3 towards the upper end of reference ranges, however this was not the primary aim of this study. Future studies might extend upon recent RMR research (Hannon et al., 2020) and concurrently examine the impact of chronic training on TH status alongside RMR to establish if current recommendations and nutritional intakes are appropriate. Similarly, a season-long study might aim to investigate variations in thyroid function across the season to find 'hot spots' in which thyroid function might be comprised, for example during pre-season or congested fixtures, or outline if there is an accumulation of effects throughout the season. In addition, although Chapter 7 outlined the acute responses to a nutritional intervention, it would be beneficial to examine the impact of chronic nutritional interventions, whether this be through prescribed nutritional intakes or through improved nutritional support, this could offer practical advice to practitioners and athletes.

Although the research presented within this thesis provides novel, free-living insight into thyroid function within these athletes, due to the number of variables and the context in which data were collected, it is difficult to establish mechanistic insight and confirm causal relationships. As such it would be useful to investigate the significant impacts on thyroid function established within this thesis in more detail, including the impact of maturation as well as nutritional intakes. Chapter 6 identified significantly lower T_4 and T_3 concentrations in those in PHV compared to those post-PHV, however no data were collected on those classified as pre-PHV. Such data would provide a better overall picture of the impact of maturation on thyroid function and the implications for nutritional support or alterations in TL. Although EA thresholds are established (Loucks et al., 2011) and initial acute data from Chapter 6 and 7 suggest that TH concentrations vary between each category, it would be beneficial to establish an EA threshold where thyroid function is compromised and establish if this aligns with current guidelines. Similarly, carbohydrate intake was shown to impact on thyroid function in these athletes, as such it would be beneficial to establish if this is a causal relationship and subsequently establish thresholds at which carbohydrate intake has detrimental impacts on thyroid function.

Injury burden is a major concern within all sports including within adolescent football with most injury incidences pertaining to contusion, sprains and growth-related injuries (Johnson et al., 2020; Materne et al., 2020; Tears et al., 2018). There are documented cases of supressed thyroid function following injury and surgery (Michalaki et al., 2001), however the impact of injury on thyroid function in adolescent athletes has not been established. Outlining the impact of injuries on thyroid function, as well as the increased injury risk with lower T_3 (Heikura et al., 2017), could provide beneficial insight into the treatment and recovery from injury, specifically into nutritional recommendations. This could be of particular interest in maturation and development injuries in which thyroid function might already be suppressed. Furthermore, although the impact of reduced thyroid function on health is established, the impact of this on athletic performance has not been researched, accordingly future studies might investigate the impact of thyroid function on performance metrics. Finally, due to the applied nature of this research it would be beneficial to establish a method of monitoring thyroid function in all athletes, as data from Chapter 5 suggests BT could provide a surrogate measure. However more data are required as to whether, if data are collected in a controlled way, if daily wake

time BT monitoring could provide a surrogate marker for thyroid function and provide information for when TH concentrations are required to be investigated further.

8.6 Concluding Remarks

This thesis has studied the TH profiles in adolescent male footballers by ascertaining natural variation in TH concentrations, identifying risk-factors associated with supressed thyroid function and finally attempting to attenuate the suppression in THs post-exercise with a carbohydrate based nutritional intervention. Combining practical assessments of THs in the free-living environment alongside free-living assessment of nutritional intake and EE has produced a thorough image of the multiple factors that impact on TH concentrations that should be considered, especially during maturation. The summaries of each study are presented above; however, the overarching theme is that there is a large individual variation in TH concentrations and as such there are limitations to reference ranges. Furthermore, the multiple stress-factors imposed on adolescent athletes could supress thyroid function and this could be mitigated through optimal nutritional and EI. Collectively, these studies progress existing scientific evidence and have contributed to knowledge around the impact of maturation, EA and nutritional intake on TH concentrations and adds to the growing body of literature of the impact of RED-S in adolescent athletes. Furthermore, this research has outlined future directions for research, as detailed above, which can provide further clarity and understanding on the implications of these findings. Moreover, the findings provide practical implications for nutritional and sports science provisions and support for adolescent footballers to promote athletic performance and health outcomes.

Chapter 9:
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Chapter 10:
Appendices

APPENDIX 1 – Example of Informed Consent Form

Northumbria University
NEWCASTLE

INFORMED CONSENT FORM: REMOVAL AND STORAGE OF TISSUE

Title of Project: The Acute Impact of Fructose and Glucose Co-ingestion on Thyroid Function Following Training and Competitive Fixtures in Elite Male Footballer Players.

Principal Investigator: Ruth Boldon
Participant Number:
I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
Blood	Analysis of thyroid hormones and cortisol concentration	Finger-tip Capillary

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

Clinical Waste [x]

Other [ ]
If other please specify.....

I consent to the University distributing this tissue to partners in this research study, outside of the University, for further testing (please tick the box if you agree). [ ]

Signature of researcher…….. Date……

Name of researcher (IN BLOCK CAPITALS)……..

Signature of participant…….. Date……

Name of participant (IN BLOCK CAPITALS)……..

Signature of parent/guardian…….. Date……

Name of parent/guardian (IN BLOCK CAPITALS)……..

Northumbria University
NEWCASTLE

INFORMED CONSENT FORM

Title of Project: The Acute Impact of Fructose Ingestion on Thyroid Function Following Training and Competitive Fixtures in Male Professional Footballer Players.

Principal Investigator: Ruth Boldon

Participant Number:

Please tick the appropriate boxes:

I have read and understand the participant information sheet.	
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	
I agree to take part in this study.	
I would like to receive individual feedback on the overall results of the study at the email address given below.	
Email address……	

Signature of researcher…….. Date……   Name of researcher (IN BLOCK CAPITALS)……..
Signature of participant…….. Date……   Name of participant (IN BLOCK CAPITALS)……..
Signature of parent/guardian…….. Date……   Name of parent/guardian (IN BLOCK CAPITALS)……..

APPENDIX 2 – Example of Participant Information Sheet

Northumbria University NEWCASTLE

The Acute Impact of Fructose and Glucose Co-ingestion on Thyroid Function Following Training and Competitive Fixtures in Elite Male Footballer Players.

Participant Information Sheet

You are being invited to take part in this research study. Before you decide it is important for you to read this leaflet so you understand why the study is being carried out and what it will involve.

Reading this leaflet, discussing it with others or asking any questions you might have will help you decide whether or not you would like to take part.

What is the Purpose of the Study

For your body to work well and for you to play well it needs to have enough energy, this comes from your 'metabolic rate' being high enough, this relies on your thyroid gland functioning correctly. Your thyroid function can change in response to high training loads and dietary intake. We are interested in looking at how an increased carbohydrate intake following intense training and competitive fixtures matches impacts on your thyroid function over the course of a standard in-season training week.

Why have I been invited?

You have been selected as you are a healthy male aged 16-23 years of age and a current player at Newcastle United Football Club. If however, you do not wish to take part or you wish to stop being part of the study then you can do this at any time.

Do I have to take part?

No. It is up to you whether you would like to take part in the study. I am giving you this information sheet to help you make that decision. If you do decide to take part, remember that you can stop being involved in the study whenever you choose, without telling me why.

What will happen if I take part?

You will be required to attend NUFC academy as normal with no alterations to scheduled training or matches. Testing will take place over the course of 2x1 week blocks. These two weeks will be selected when you have one home fixture within the 7day period and regular training throughout the other days. These two weeks may be consecutive or as close together as possible in order to make testing as easy as possible for yourself.

During each 7 day period you will be asked to write down all your food and drink in a **food diary** and **wear a small accelerometer around your waist** when not training or playing, these combined will monitor your energy intake and energy expenditure. During training and matches you will wear your **GPS monitor** as normal to assess training load.

On each day you have training or a match the researcher will conduct a few tests before and after each session, these should take approximately 10minutes and will not interfere with your training. These tests are:

Body temperature: a small digital thermometer will be held in your armpit for 2minutes.
Blood pressure: a digital wrist based monitor will be used to measure your blood pressure.
Thyroid hormones and cortisol: A finger-tip blood sample of approximately 0.5ml of blood will be taken.
Food recall: A few questions asked based on your food intake from the previous day.

What are the possible benefits of taking part?

By taking part in this study you will give us a greater insight into how your thyroid function changes in response to training loads and nutritional intake. You will help us to develop specific interventions to improve thyroid function around match and intense training sessions.

What are the possible disadvantages of taking part?

You may feel slight pain when a small pinprick is made in your finger-tip to allow for blood sampling and you may feel a small amount of pressure applied to the finger but this is only brief. The blood pressure may feel momentarily tight around your wrist but this is released promptly.

Will my taking part in this study be kept confidential and anonymous?

Yes. Your name will not be written on any of the data we collect; the written information you provide will have an ID number, not your name, and your name will not appear in any reports or documents resulting from this study. The consent form you have signed will be stored separately from your other data. The data collected from you in this study will be confidential. The only exception to this confidentiality is if the researcher feels that you or others may be harmed if information is not shared.

Can I withdraw from the project?

You have the right to withdraw from the study at any point, if you decide you no longer wish to take part in the study then please inform the principal investigator Ruth Boldon by email: r.boldon@northumbria.ac.uk

How will my data be stored, and how long will it be stored for?

All paper data, including your consent forms will be kept in locked storage. All electronic data will be stored on the University U drive, which is password protected. All data will be stored in accordance with University guidelines and GDPR.

What categories of personal data will be collected and processed in this study?

Biometric and health data will be collected as part of this research.

What is the legal basis for processing personal data?

The legal basis for processing the personal data required for the purposes of this study is that the research is necessary for scientific and historical research purposes"

Who are the recipients or categories of recipients of personal data, if any?

The research team at Northumbria will be the only recipients of the personal data from this study.

What will happen to the results of the study and could personal data collected be used in future research?

The general findings will be used within a PhD thesis and might be reported in a scientific journal or presented at a research conference, however the data will be anonymized and you or the data you have provided will not be personally identifiable, unless we have asked for your specific consent for this beforehand. The findings may also be shared with NUFC academy. We can provide you with a summary of the findings from the study if you email the researcher at the address listed below.

Who is Organizing and Funding the Study?

Northumbria University and Newcastle United Football Club jointly organize and fund this study.

Northumbria University
NEWCASTLE

Who has reviewed this study?

The Department of Sport, Exercise and Rehabilitation Research Ethics Committee at Northumbria University have reviewed the study in order to safeguard your interests, and have granted approval to conduct the study.

What are my rights as a participant in this study?

You have a right of access to a copy of the information comprised in their personal data, a right in certain circumstances to have inaccurate personal data rectified; and a right to object to decisions being taken by automated means. If you are dissatisfied with the University's processing of personal data, you also have the right to complain to the Information Commissioner's Office.

Contact for further information:

Researcher email: r.boldon@northumbria.ac.uk
Supervisor email: mick.wilkinson@northumbria.ac.uk

Name and contact details of the Data Protection Officer at Northumbria University: Duncan James (dp.officer@northumbria.ac.uk).