Associations of Thyroid Stimulating Hormone Concentrations within the Normal Reference Range on Energy Expenditure, Macronutrient Oxidation and Body Composition

By

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CERTIFICATE OF APPROVAL

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List of Abbreviations

BMD	Bone Mineral Density
BMI	Body Mass Index
CV	Coefficient of Variation
DEXA	Dual-Energy X-ray Absorptiometry
FT3	Free Triiodothyronine
FT4	Free Thyroxine
FFM	Free-Fat Mass
FM	Fat Mass
HIPPA	Health Insurance Portability and Accountability Act
L-T4	Levothyroxine
MIT	Monoiodotyrosine
NHANES	National Health and Nutrition Examination Survey
OCTRI	Oregon Clinical and Translational Research Institute
OHSU	Oregon Health & Science University
PAEE	Physical Activity Energy Expenditure
REE	Resting Energy Expenditure
RQ	Respiratory Quotient
T3	Triiodothyronine
T4	Thyroxine
TBW	Total Body Water
TEE	Total Energy Expenditure
TEF	Thermic Effect of Food
TSH	Thyroid Stimulating Hormone
UUN	Urine Urea Nitrogen

ABSTRACT

Patients with overt thyroid disease have altered metabolic function. It is not clear whether metabolic function varies with normal thyroid function as measured by serum thyroid stimulating hormone (TSH) concentration. To address this gap in clinical knowledge, a cross-sectional study was carried out to determine the relationship between serum TSH concentrations within the normal range and markers of body composition, energy expenditure, and macronutrient oxidation in healthy control and euthyroid L-T4 treated women.

Participants (n=65) were divided into low-normal (0.34 - 2.49 mU/L, n = 46) and high-normal (2.50 – 5.60 mU/L, n = 19) TSH concentration groups. Total energy expenditure by doubly labeled water, resting energy expenditure and thermic effect of food by indirect calorimetry, and body composition by DEXA were measured. Macronutrient oxidation rates were calculated using standard equations. Means and differences in means of body composition and energy expenditure variables and macronutrient oxidation rates between groups were compared using independent t-tests and linear regression models and across TSH as a continuous variable.

The average weight was significantly lower in the low-normal than the high-normal TSH group (72.7 ± 15.3 vs. 82.2 ± 22.3 kg, p=0.05) as was BMI (27.1 ± 5.4 vs. 30.5 ± 8.0 $kg/m^{2,}$ p=0.05). No other differences between groups or relationships with TSH were observed. In conclusion, we found no associations between variations in TSH concentration within the established normal range and differences in body composition, energy expenditure or macronutrient oxidation parameters.

Chapter 1: Significance

Patients with overt thyroid disease are known to have alterations in metabolic function, but it is not clear whether variations in thyroid function within the normal reference range affect metabolism. In the field of endocrinology, controversy centers around treatment of mild hypothyroidism which incorporates both mild elevations in thyroid stimulating hormone (TSH) and the extension of this potential diagnosis to the high-normal reference range of TSH. Mild elevation in TSH, also referred to as subclinical hypothyroidism, is defined as an elevated serum TSH concentration (~4.5-10.0 mU/L) with normal free thyroxine (FT4) (0.7-1.8 ng/dl) and free triiodothyronine (FT3) (2.4-4.2 pg/mL) concentrations. Most studies define the “normal” serum TSH reference range between the lower normal serum TSH concentration of about 0.3 mU/L to the normal upper serum TSH concentration of about 5.0 mU/L (1-2).

Recent U.S. population-based data show that 2.5% of the adult population has mildly elevated TSH concentrations, and 14% has high-normal TSH concentrations (1), for a total of over 50 million affected individuals. Emerging data suggests that patients with TSH concentrations between 2.5 - 4.5 mU/L are more likely to have anti-thyroid peroxidase antibodies (anti-TPO Ab), a surrogate marker for thyroid disease, showing that these two ranges of thyroid function are not distinct, but represent a continuum of mild hypothyroidism. Figure 1. summarizes the serum TSH concentration reference range and illustrates the continuum of mild thyroid dysfunction that extends down into the upper normal serum TSH range. It is controversial to treat patients with only mildly elevated TSH concentrations, much less those with TSH concentrations in this range (2.5 – 5.0 mU/L) since data regarding whether these very mild thyroid alterations lead to clinically relevant end-organ effects are limited and contradictory.

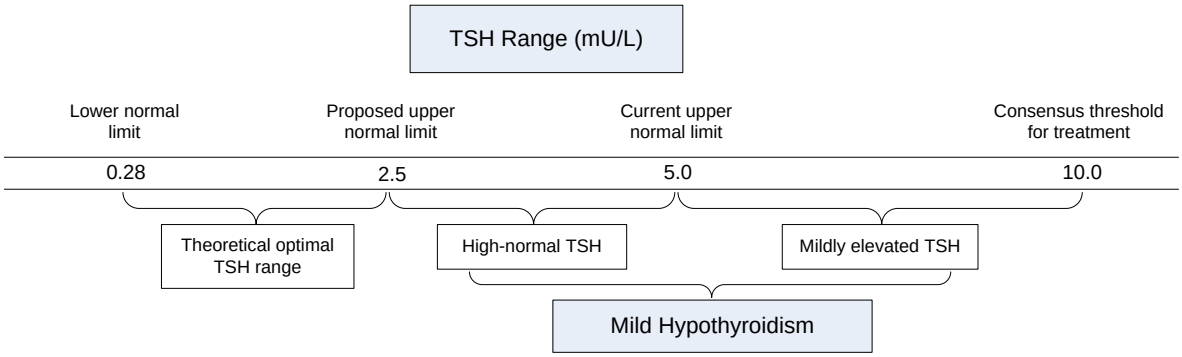

Figure 1. Serum TSH Concentration Continuum

Adverse health consequences of mild thyroid dysfunction have not been clearly determined, but include an increased risk for progression to overt hypothyroidism (2). Progression from mild to overt hypothyroidism is reported to vary from 5% to 26% per year (3). Overt hypothyroidism is associated with metabolic abnormalities such as lower resting energy expenditure, hyperlipidemia, and alterations in body composition (4). Much less is known about the clinical consequences of high-normal TSH concentrations which are more common, as well as the benefits and disadvantages of treating mild hypothyroidism .

Thyroid hormone has long been known to affect metabolic processes. Within the normal range of thyroid function, there is a positive association between serum TSH concentrations and body mass index, percent body fat, and basal oxygen consumption; a positive association between free triiodothyronine (FT3) concentrations and resting energy expenditure (REE), sleeping metabolic rate and 24-hour lipid oxidation rate; and a negative association between FT3 concentrations and 24-hour respiratory quotient (RQ)(5-8). In healthy humans, triiodothyronine (T3) concentrations account for 1-2% of the variation in total energy expenditure and 20-25% of REE (9), enough to explain variations in weight gain and body composition over time. Variations in thyroid function within the normal range may exert significant effects on energy expenditure and macronutrient oxidation to concentrations that are relevant for variation in body composition.

Study Aims and Hypotheses

To provide additional evidence of the physiological consequences associated with serum TSH concentrations within the upper normal range, and to contribute to the data supporting a clinically relevant definition of mild hypothyroidism, this study determined the relationship between serum TSH concentrations within the established normal range (0.34 – 5.60 mU/L) and measurements of body composition, energy expenditure, and macronutrient oxidation in healthy control and euthyroid L-T4 treated women. We hypothesized that energy expenditure and fat oxidation rates would be higher and body fat percentage and protein and carbohydrate oxidation rates would be lower among women with serum TSH concentrations within the low-normal range (0.34 - 2.49 mU/L) compared to women with serum TSH concentrations within the high-normal range (2.50 – 5.60 mU/L).

Chapter 2: Background

Overview of Hypothyroidism

The human thyroid gland is a five centimeter long, butterfly-shaped gland that is located at the front of the neck just below the larynx. The thyroid gland is a component of the endocrine system that produces, stores and releases hormones into the circulation to regulate many physiological functions. In particular, the thyroid gland produces and secretes thyroid hormones. Thyroxine (T4) is the main product of the thyroid gland. T4 can be converted to its active form, triiodothyronine (T3) and binds to T3 receptors that alters transcription of genes in target organs including the liver, kidney, pituitary, heart, brain, spleen and testis (10). Decreased production and secretion of T4 and T3 by the thyroid gland and increased production of thyroid stimulating hormone (TSH) by the pituitary gland characterize the most common type of thyroid dysfunction, hypothyroidism.

There is a log-linear relationship between TSH and T4 concentrations, such that very small changes in T4 concentrations that move away from an individual's set point lead to large reciprocal changes in TSH concentrations. This occurs even when the T4 and T3 concentrations remain within the population normal range. A pattern of abnormal TSH concentrations with normal T4/T3 concentrations is termed "subclinical" thyroid disease. There are two categories of subclinical thyroid disease: subclinical hypothyroidism (isolated elevated TSH), and subclinical hyperthyroidism (isolated decreased TSH). For the work presented here, we focused specifically on TSH concentrations in the low-normal range (0.34 – 2.5 mU/L) and in the high-normal TSH range (2.5-5.60 mU/L).

Two recent studies in large numbers of free-living subjects highlight the high prevalence of mild hypothyroidism (11,12). In these studies, 4-5% of younger subjects and up to 20% of older women had subclinical hypothyroidism, with 75% having mildly elevated TSH concentrations within 5-10 mU/L (11). In the Colorado Thyroid Disease Prevalence Study (11), an astonishing 40% of subjects taking the thyroid hormone (levothyroxine (L-

T4)) had abnormal TSH concentrations, half with low concentrations and half with elevated concentrations. Overall population data report that 2.5% of the U.S. adult population who are not already receiving L-T4 therapy, or 7.5 million people, have mild subclinical hypothyroidism (1).

The normal TSH reference range does not follow a normal distribution curve. It is skewed at the upper range, suggesting that this range may include subjects who are not "truly normal" as shown in Figure 2. The normal reference range includes a small, but significant number of subjects with anti-thyroid peroxidase antibodies (anti-TPO Ab) which are a marker of auto-immune thyroid disease. In the NHANES III population, healthy subjects with no history of thyroid disease who had serum TSH concentrations in the high-normal range had higher rates of anti-TPO Ab, compared to healthy subjects with TSH concentrations below 1.5 mU/L(1,12). When anti-TPO positive subjects were removed, the TSH upper normal limit decreased to 3.5 mU/L. This suggests that normal TSH data is skewed by subjects who have developing stages of autoimmune hypothyroidism, with higher TSH concentrations than optimal for their endogenous set-points. Further normalization of the TSH reference range to a normal distribution curve leads to the theoretical upper normal limit of 2.5 mU/L shown above (solid line), which is well below current laboratory cut-off points. Using this logic displayed in Figure 2, theoretically, 17% of the normal or "euthyroid" United States population, approximately 51 million people, would be diagnosed with "low-normal thyroid function," based on a cut-off point of serum TSH concentration above 2.5 mU/L(1).

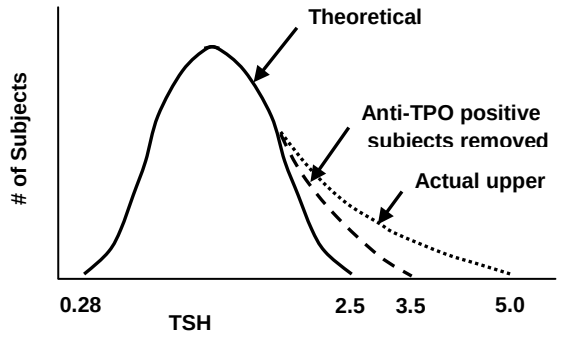

Figure 2: The Theoretical Normal Serum TSH Reference Range

Clinical Relevance of Mild Hypothyroidism and Controversy of Treatment

These data sparked widespread debate within the thyroid field as to whether "euthyroid" subjects with TSH levels above 2.5 mU/L are truly normal, and whether hypothyroid subjects are undertreated if their serum TSH concentrations are above 2.5 mU/L. Two editorials written by prominent thyroid specialists illustrate this controversy: "The thyrotropin reference range should remain unchanged" (2), and "The evidence for a narrower thyrotropin reference range is compelling" (13). Surks et al., estimated from the reference group of NHANES III, that if the upper limit of the serum TSH reference range were to decrease from ~4.5 mU/L to 2.5 mU/L about 22-28 million Americans would be considered hypothyroid (2). Surks et al. suggested that treating individuals with TSH concentrations between 2.5 mU/L to 4.5 mU/L would lead to overtreatment and risk inducing exogenous subclinical hyperthyroidism (2). These investigators concluded that since levothyroxine (L-T4) is not recommended for treatment of subclinical hypothyroidism, treatment certainly is not recommended for individuals with a serum TSH concentration trending towards the upper limit of the normal reference range (2). However, investigators recommend that these individuals should have serum TSH concentrations checked every 1-2 years to determine if they have developed hypothyroidism (2).

In contrast, Wartofsky et al. showed that serum TSH concentrations between 2.5 mU/L to 4.5 mU/L allowed for early detection of subclinical thyroid dysfunction and concluded that patients with a serum TSH in this range could benefit from closer follow-up (13). These authors concluded that 95% of the US population had serum TSH concentrations <2.5 mU/L and recommended that individuals with TSH concentrations above this level should receive careful assessment and treatment (13).

Clinical Manifestations of Subclinical Hypothyroidism

Subclinical hypothyroidism encompasses a broad range of symptoms and descriptive terms. Signs of hypothyroidism include slow movements, slow speech, hoarseness, bradycardia, nonpitting edema, hyporeflexia, and delayed relaxation of reflexes (14). Symptoms of hypothyroidism include fatigue, lethargy, sleepiness, mental impairment, depression, cold intolerance, dry skin, decreased perspiration, weight gain, decreased appetite, constipation, menstrual disturbances, arthralgia, and paresthesia (14). Serum TSH concentrations >10 mU/L have been linked to detrimental effects such as accelerated atherosclerosis, fatigue and dyspnea, CO_2 retention, and alterations in systemic hemodynamics and neural and somatic growth and development (15).

Important target organs for thyroid hormone action include the brain, liver, skeletal muscle, heart and bone. These organ systems should be studied in the normal TSH reference range to see if they are also affected. Thyroid hormones play a role in energy expenditure and impairment of thyroid function is thought to contribute to decreased energy expenditure which may contribute to the development of many of the signs and symptoms listed above (16) Metabolic outcomes of low-normal and high-normal serum TSH concentrations were studied to better understand these clinical consequences.

Impact of Thyroid Function on Energy Expenditure (Appendix 1)

It is well established that patients with overt hypothyroidism have lower rates of energy expenditure than those who are euthyroid. Decreased energy expenditure may contribute to the physical and behavioral symptoms of hypothyroidism such as feeling weak, lethargic, fatigued and sensitive to cold temperatures. Reduced energy expenditure influences the regulation of body weight and gradual weight gain is a common symptom of hypothyroidism. In healthy adults, total energy expenditure (TEE) is the sum of energy

expended while at rest to maintain normal physiological function (Resting Energy Expenditure, REE), energy expended to support physical activity (PAEE), and energy expended to digest and metabolize food (Thermic Effect of Food, TEF). Total energy expenditure can be measured as a whole or as the sum of its parts.

The association between total energy expenditure and normal reference range serum TSH concentrations has not been well studied. However, Rondeau et al, evaluated total energy expenditure through a cross-sectional study of 104 overweight and obese, postmenopausal, euthyroid women. Total energy expenditure was determined using the doubly labeled water method over a 10-day period. Women were stratified into three groups by plasma TSH concentration: group 1 (TSH 0.34-1.66 mU/L, n= 34), group 2 (TSH 1.67-2.91 mU/L, n=35), group 3 (TSH 2.04-4.84, n=35). Total energy expenditure and plasma TSH concentrations were negatively correlated, but the relationship not statistically significant ($r = -0.141$, $p = 0.328$) and total energy expenditure did not vary significantly between the three groups (group 1: 2530 ± 372, group 2: 2509 ± 447, group 3: 2408 ± 378 kcal/d)(4).

Resting Energy Expenditure

Resting energy expenditure (REE) is the energy required to maintain the body's basic metabolic activity and organ functions while at rest. Such activities include respiration, cardiac function, and maintenance of normal muscle tension and regulation of body temperature. REE accounts for about 60% of total energy expenditure in sedentary individuals (17). REE is best assessed when an individual has fasted for a minimum of 5 hours, has refrained from physical activity for 12 hours, and has abstained from nicotine, caffeine and other stimulants for 24 hours (18). Indirect calorimetry is the gold standard method for assessing REE and has been used to assess REE in individuals with hypothyroidism (18).

Tagliaferri et al. showed that resting energy expenditure was inversely related to TSH concentrations in 108 obese patients (average BMI, 43.4 ± 6.6 kg/m^2) with subclinical hypothyroidism (19). Subclinical hypothyroidism was defined as normal FT4 concentrations and a serum TSH concentration >4.38 mU/L (average TSH, 6.4 ± 2.7 mU/L). Obese, hypothyroid patients were compared to a group of 131 obese (average BMI, 42.9 ± 6.8 kg/m^2) controls with normal serum TSH concentrations (2.1 ± 1.1 mU/L) matched for age, sex, and BMI. A 7-day dietary record was obtained to assess daily energy intake (2843 ± 1386 kcal/24 h vs. control, 3148 ± 1551 kcal/24 h), indirect calorimetry was performed to measure REE (1826 ± 362 kcal/24 h vs. control, 1821 ± 324 kcal/24 h), bioelectrical impedance analysis (BIA) was used to measure free-fat mass (FFM, 53.9 ± 6.6% of total mass vs. control 55.1 ± 5.9% of total mass). None of the means of these metabolic parameters were significantly different between the patients with subclinical hypothyroidism and the controls. When patients were stratified into subgroups based on serum TSH concentrations and when energy expenditure was indexed to fat free mass for comparison purposes, 63% of the subclinical hypothyroid patients had TSH <5.7 mU/L and had an estimated energy expenditure of ~31 kcal/kg FFM. Thirty-seven percent of subclinical hypothyroid patients had a TSH >5.7 mU/L and had an estimated energy expenditure of ~28 kcal/kg FFM ($p<0.05$). The authors concluded that subclinical hypothyroidism affected REE in obese patients when serum TSH concentrations were above the normal range (>5.7 mU/L) (19).

In a prospective study, Boeving et al., compared the effects of serum TSH concentrations on REE. Forty-two patients with newly diagnosed overt hypothyroidism (TSH >10 mUI/L) were treated with varying doses of L-T4 to achieve and sustain TSH concentrations within a low-normal TSH range (0.4-2.0 mIU/L, n=20) or a high-normal TSH range (2.0-4.0 mIU/L, n=22 (20)). After the target TSH range was reached, patients were

evaluated every 3 months for thyroid function, REE, body composition and bone mineral density for 12 months. There was a significantly greater ($p=0.02$) relative increase in REE from the initial visit to the final visit in the low-normal TSH range group (7.1% ± 11.3%) compared to the high-normal TSH range group (3.6 % ± 15.1%) suggesting that treatment with L-T4 to achieve low-normal serum TSH concentrations was associated with an increase in REE (20).

Al-Adsani et al. investigated the effects of varying doses of levothyroxine (L-T4) on serum TSH concentrations and REE in nine subjects treated for hypothyroidism. Each patient received three successive doses of L-T4, one to achieve normal TSH concentration, the second to achieve a slightly reduced TSH concentration and the third to achieve a slightly elevated serum TSH concentration over a 6-8 week period. Doses were increased or decreased between 25 and 50 µg/day based on the initial serum FT4 and TSH concentrations of the patient. The daily dose varied between ~15-66% above or below the patient's initial L-T4 dose to shift the serum TSH concentration below or above the normal range, respectively. Resting energy expenditure was measured by indirect calorimetry, fat-free mass (FFM) was measured by BIA and the ratio of REE/kg FFM was calculated for comparison purposes. When higher doses of L-T4 were administered, serum TSH concentrations varied between 0.05-1.40 mU/L and REE increased by 17-220 kcal/24 h in patients with TSH concentrations within the normal range (21). When lower L-T4 doses were administered, serum TSH concentrations varied between 0.42-18.0 mU/L and REE decreased in every patient by 75-150 kcal/24 h (21). Overall, when serum TSH concentrations varied between 0.1-10 mU/L, REE decreased by 15%, indicating sensitivity of REE to small changes in thyroid function (21).

Physical Activity Energy Expenditure

Physical Activity Energy Expenditure (PAEE) is the energy expended during low-intensity daily activities, short bursts of high-intensity activities, and unintentional activity,

such as fidgeting. PAEE is the most variable component of total energy expenditure. In sedentary individuals, PAEE accounts for about 15% of total energy expenditure (17). However, in active individuals, PAEE can account for >50% of total energy expenditure (17). PAEE is difficult to measure directly and is often estimated as the difference between total energy expenditure and the sum of measured resting energy expenditure plus thermic effect of food.

The association between PAEE and normal reference range of serum TSH concentrations has not been extensively studied. However, one study by Caraccio et al. reported that energy expenditure and substrate oxidation response to exercise was lower in participants with subclinical hypothyroidism compared to controls (22). This study used a double-blind, randomized, placebo-controlled trial to evaluate response of total energy expenditure and substrate oxidation to exercise in participants treated with L-T4 for subclinical hypothyroidism.

The 23 participants with subclinical hypothyroidism were characterized by elevated serum TSH concentrations (>3.6 mU/L; average TSH concentration, 4.65 ± 0.20 mU/L) and were treated with 25 µg of L-T4, twice daily for 12 months. Participants were matched to 10 euthyroid controls (average TSH concentration, 1.39 ± 0.18 mU/L) treated with a placebo for 6 months. Participants were involved in an incremental step-up cycling exercise protocol that measured oxygen consumed (VO_2), CO_2 produced (VCO_2), and heart rate. Blood glucose, lactate, pyruvate, free fatty acid, glycerol, and β-hydroxy-butyrate concentrations were measured before exercising, every 2 minutes during exercise, and during recovery. The exercise protocol was repeated after 6 months of placebo supplementation or 6 and 12 months of L-T4 treatment. The authors reported that despite treatment with L-T4 for 6 months, maximal power output (80 ± 8 watts, vs. control, 88 ± 13 watts, p=0.02) and maximal oxygen uptake (39.8 ± 2.4 mL/min/kg, vs. control, 41.9 ± 2.4 mL/min/kg, p=0.04) were lower in participants with subclinical hypothyroidism compared to euthyroid controls.

During the exercise protocol, the respiratory quotient (RQ), a measure of relative fat, carbohydrate and protein oxidation, increased in all participants suggesting that the rate of carbohydrate oxidation increased, and/or the rate of fat oxidation decreased, however the change in RQ from pre-exercise was significantly greater in the subclinical hypothyroid group, from 0.8 to 1.0 than in the controls from 0.82 to 0.90 ($p=0.04$). Higher serum TSH concentrations were directly correlated with higher blood lactate concentrations suggesting an increased concentration of hydrogen ions resulting in possible increased acidity (22).

Despite these differences at 6 months, after 12 months of L-T4 treatment, there were no significant changes in metabolic response to exercise among subclinical hypothyroid participants compared to baseline or compared to controls (22). Investigators concluded that patients with subclinical hypothyroidism had an impaired metabolic response to exercise that was not corrected by restoring serum TSH concentrations to the normal reference range with L-T4 replacement over 6-12 months (22).

Thermic Effect of Food

Thermic effect of food (TEF) is the amount of energy expended to digest food and to absorb, transport, and store nutrients consumed. TEF is measured by indirect calorimetry and is calculated as the total energy expended during the post-prandial period (typically 4-6 hours after meal consumption) minus the resting energy expenditure measured just prior to meal consumption. TEF reflects meal-induced thermogenesis during the postprandial period and accounts for about 10% of total energy expenditure. TEF is affected by energy content and macronutrient composition of test meals and activity performed before eating a meal such that increased energy intake results in increased post-prandial energy expenditure and lower energy intake results in lower postprandial energy expenditure (23,24). Macronutrient content of a meal affects duration of TEF. Protein-rich meals result in a higher and longer postprandial energy expenditure than carbohydrate-rich meals (25). TEF is also higher in

healthy adults who habitually exercise compared to adults who are sedentary (23). Bielinski et al., determined that TEF was 9% higher when a standardized meal was consumed after 3-hours of walking compared to no prior exercise (26). To date, no studies of the relationship between TEF in patients with serum TSH concentrations within the normal reference range have been published. This gap in knowledge is addressed by the study reported herein.

Macronutrient Oxidation

Energy is derived through the oxidation or the breakdown of food substrates known as macronutrients (carbohydrates, fats, and proteins). Macronutrients are carbon-based molecules that can be broken down into carbon dioxide (CO_2) and water (H_2O) with the release of energy in the form of heat when oxygen (O_2) is present. Macronutrient oxidation is estimated by indirect calorimetery through which the volume of O_2 consumed and the volume of CO_2 produced is measured at a given time. This information, along with urine urea nitrogen (UUN), is entered in prediction equations to calculate the rates of carbohydrate, protein, and fat oxidized:

Carbohydrate Oxidation (g/min) = 4.59 (VCO_2, L/min) - 3.25 (VO_2, L/min - 3.68 (UUN, g/min)

Fat Oxidation (g/min) = 1.69 (VO_2, L/min) - 1.69 (VCO_2, L/min) - 1.72 (UUN, g/min)

Protein Oxidation (g/min) = 6.25 g Protein / g Nitrogen (UUN, g/min)

Respiratory Quotient

Respiratory quotient (RQ) is a relative measure of macronutrient oxidation and is defined as the ratio of the volume of carbon dioxide (VCO_2) produced divide by volume of oxygen (VO_2) consumed, or VCO_2/VO_2, while at rest. RQ typically varies from 0.67 to 1.2 and reflects the relative amount of protein, fat, and carbohydrate oxidized at a given time. As

illustrated in the following equations, when 1 gram of carbohydrate is oxidized, 0.746 L of O_2 are consumed and 0.746 L of CO_2 are produced yielding a RQ of 1.0. In contrast, when 1 g of fat is oxidized 2.03 L of O_2 are consumed and 1.43 L of CO_2 are produced yielding an RQ of 0.70.

$$1\text{ g carbohydrate} + 0.746\text{ L } O_2 \rightarrow 0.746\text{ L } CO_2 + 0.6\text{ g } H_2O$$

$$1\text{ g fat} + 2.03\text{ L } O_2 \rightarrow 1.43\text{ L } CO_2 + 1.09\text{ g } H_2O$$

$$1\text{ g protein} + 0.966\text{ L } O_2 \rightarrow 0.782\text{ L } CO_2 + 0.45\text{ g } H_2O + 0.16\text{ g N (27)}$$

For example, carbohydrate in the form of glucose ($C_6H_{12}O_6$) is oxidized to 6 moles of H_2O and 6 moles of CO_2 as represented in the following formula:

$$\text{Glucose } (C_6H_{12}O_6) + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2\ (28)$$

In this process, 6 moles of O_2 are consumed and and 6 moles of CO_2 produced for each mole of glucose oxidized. Thus, the RQ, or the ratio of VCO_2/VO_2, is 1.0 because the amount of CO_2 produced is equal to the amount of O_2 consumed (28). In contrast, when a typical triglyceride (palmitoyl-stearoyl-oleoyl-glycerol, PSOG, $C_{55}H_{104}O_6$) is oxidized, the amount of CO_2 produced (55 moles) is lower than the amount of O_2 consumed (78 moles) and the VCO_2 to VO_2 ratio of 55 moles of CO_2/ 78 moles of O_2 is closer to 0.7.

$$\text{PSOG } (C_{55}H_{104}O_6) + 78\ O_2 \rightarrow 55\ CO_2 + 52\ H_2O\ (28)$$

A respiratory quotient value of 1.0 indicates high whole-body carbohydrate oxidation and low whole-body fat oxidation and is a predictor of weight gain which will be discussed further in the weight gain and thermogenesis section below (6). Because most meals are comprised of a combination of macronutrients when a mixed meal is consumed the RQ is typically around 0.86 (28).

Impact of Thyroid Function on Macronutrient Oxidation

Thyroid hormones have both hyper- and hypoglycemic effects which influence metabolism. Thyroid hormones stimulate glycogenolysis, gluconeogenesis, anabolism, and lipogenesis while simultaneously stimulating glycolysis, catabolism, and lipolysis. Thyroid hormones increase sensitivity of the sympathetic nervous system to mediate lipolysis and increase fat oxidation by stimulating the expression of carnitine palmitoyl transferase, an enzyme required for fatty acid transport into the mitochondrial matrix for metabolism via β-oxidation (6).The effect of thyroid hormones on lipolysis is more prominent than its effect on lipogenesis.

Although no studies have been reported on the association between thyroid function and macronutrient oxidation, a study by Buemann et al. demonstrated that women who were formerly obese and prone to weight gain had lower T3/T4 ratios after consuming a high fat diet than control subjects suggesting an altered thyroid response to dietary composition (29). In this study, energy expenditure and substrate oxidation rates in eight formerly obese women were compared to eight control women. Measurements were taken over four consecutive days while the women were housed in a whole room respiratory chamber. On the first and fifth day of the study, each subject consumed a diet comprised of 30% of energy from fat, 55% from carbohydrate and 15% from protein. On the intervening days, subjects consumed a diet comprised of 55% of energy from fat, 30% of energy from carbohydrate, and 15% from protein. Blood FT4 and FT3 concentrations were measured before the first high fat meal and again 3 days later. Women in the formerly obese group had a lower mean total energy expenditure (1981 ± 48 kcal/day) compared to women in the control group (2104 ± 58 kcal/day) while consuming the high fat diet, but the difference was not statistically significant ($p<0.13$). Plasma free T3/T4 ratios decreased in the formerly

obese women after consuming a high fat diet (from 0.26 ± 0.02 to 0.19 ± 0.01 pmol/L, $p<0.005$) and positively associated with total energy expenditure. (29). Plasma triglyceride concentrations also decreased significantly in the formerly obese group (0.93 ± 0.13 to 0.74 ± 0.07 mmol/L, $p<0.005$) than the control group (1.44 ± 0.21 to 1.06 ± 0.13 mmol/L) after consuming the high fat diet. There was a greater increase in RQ in the formerly obese group (+0.053 ± 0.009) than the control group (+0.030 ± 0.005, $p=0.02$) after consuming the high fat diet suggesting a lower rate of fat oxidation (29).

Influence of Hypothyroidism on Body Weight and Body Composition

Thyroid hormones play a role in weight regulation and impaired thyroid function is thought to contribute to the pathogenesis of obesity (16). Obesity is a disease that results when the amount of energy consumed exceeds the amount of energy expended; which when maintained overtime, results in excess energy stored as fat. Hypothyroidism plays a significant role in weight gain as thyroid hormones stimulate thermogenesis, the release of energy in the form of heat. Impaired thermogenesis in response to certain stimuli is associated with weight gain and obesity. Predictors of weight gain include a lower TEE, a lower REE, a lower TEF, and a higher RQ (6). The thermogenic effect of thyroid hormones is related to increased appetite and synthesis and deposition of fat. In individuals who are hypothyroid, synthesis and mobilization of fat is reduced as a result of lower lipogenic enzyme activity, specifically carnitine palmitoyl transferase (6). Weight gain occurs when the mobilization of fat stores is reduced and serum fatty acid concentrations, in the form of triglycerides, increase (6).

A study by Knudsen et al. described the association between thyroid function and body mass index (BMI) in a Danish population. This study was conducted among 4649 participants without previous or current overt thyroid dysfunction. Participants were stratified into five subgroups based on serum TSH concentrations: group 1 (TSH <0.4 mU/L), group 2

(TSH 0.4 - 0.99 mU/L), group 3 (TSH 1.0 - 1.99 mU/L), group 4 (TSH 2.0 - 3.6 mU/L), and group 5 (TSH >3.6 mU/L). At follow-up after 5 years, the difference in BMI between group 1 and group 5 was 1.9 kg/m^2 reflecting a significant difference in weight gain of 5.5 kg (5).

A study by Svare et al. described the longitudinal relationship between serum TSH concentration and body composition and the associations between change in weight and change in serum TSH concentration after 10.5 years of follow-up. A total of 9954 women and 5066 men without self-reported thyroid disease and with serum TSH concentrations between 0.5-3.5 mU/L at baseline were studied. At follow-up, mean weight was 1.8 kg (95% CI 1.7, 1.9) higher and mean BMI was 1.0 kg/m^2 (95% CI 1.0, 1.1) higher in women than at baseline. Mean waist circumference and waist-to-hip ratio was also higher in women at follow-up by 10.5 cm (95% CI 0.9, 1.0) and 0.09 cm (95% CI 0.09, 0.09), respectively (30). Positive associations were seen between body composition measurements and serum TSH concentrations. As serum TSH concentrations increased by 1.0 mU/L, body weight increased by 0.9 kg, BMI increased by 0.3 kg/m^2, and waist circumference increased by 0.6 cm (30). A weight gain of more than 5 kg was associated with an increase in serum TSH concentration of 0.08 mU/L. In contrast, serum TSH concentrations decreased by 0.12 mU/L among women whose weight decreased by 5 kg (30). The authors concluded that changes in TSH concentration correlates with changes in measurements of weight. The researchers also confirmed that weight gain is accompanied by increased serum TSH concentrations and weight loss is associated with decreased serum TSH concentrations (30).

A longitudinal study by Ortega et al. investigated the associations between thyroid hormone concentration and obesity in euthyroid adult Pima Indians (n=89). In this study, the normal serum TSH concentration range was defined as 0.6-4.6 mU/L. Baseline FT3 concentrations were positively associated with absolute and annual percentage change in weight after 4 ± 2 years of follow-up (p=0.02, p=0.009). Baseline plasma TSH

concentrations were positively associated with body weight ($p \leq 0.01$) and percent body fat ($p<0.01$), but not with FT3 or FT4 concentrations (6). The authors concluded that lower FT3 concentrations at baseline, but not FT4 concentrations, predicted weight gain (6).

In a prospective, interventional study, 42 patients with newly diagnosed overt hypothyroidism who were treated with L-T4 were stratified into two groups based on serum TSH concentrations: low-normal serum TSH (0.4 - 2.0 mU/L, n=20) and high-normal serum TSH (2.0 - 4.0 mU/L, n=22) (20). Patients were evaluated every 3 months for thyroid function, resting energy expenditure, body composition and bone mineral density over a 12 month period. Patients with a BMI >35 kg/m^2 were excluded. Total body composition including fat mass, lean mass (LM), and bone mass was measured by DEXA and resting energy expenditure was measured by indirect calorimetry.

At baseline, the average BMI of the low-normal TSH group was 27 ± 5 kg/m^2 and the average serum TSH concentration was 58.9 ± 47.6 mU/L before L-T4 treatment (20). The baseline BMI of the high-normal group was 29.3 ± 4 kg/m^2 and the average serum TSH concentration before L-T4 treatment was 44.6 ± 26 mU/L. In the low-normal group, REE was 29.5 ± 2.9 kcal/kilogram lean mass/day compared 30.4 ± 5.3 kcal/kilogram lean mass /day in the high-normal group at baseline and was not statistically significant (p=0.5) (20). Lean mass was 41.8 ± 7.3 kg in the low-normal group compared to 42.7 ± 9.0 kg in the high-normal group at baseline (p=0.6). Total fat mass in both groups combined increased from 25.9 ± 8.5 to 26.2 ± 9.6 kg (p=0.02) while lean mass decreased from 42.3 ± 8.2 kg to 42.0 ± 7.3 kg (p=0.001) after 12 months of L-T4 treatment. BMI and bone mineral density did not change or vary between groups (20).

Based on these studies, serum TSH concentrations within the normal reference range are associated with energy expenditure, macronutrient oxidation and body

composition. The study described here will expand on these findings and will be the first to focus specifically on the simultaneous and synergistic cross-sectional relationship of serum TSH concentrations within the normal range (0.34 – 5.60 mU/L) on energy expenditure, macronutrient oxidation, and body composition measurements. This research provides critical data to better define the mechanisms underlying clinically relevant effects of TSH concentrations within normal reference range and will contribute evidence needed to establish appropriate therapeutic TSH reference ranges.

Chapter 3: Methods

General Study Design and Setting

This study was a cross-sectional analysis of data obtained as a part of "The Effects of Mild Hypothyroidism and Variations in Thyroid Function within the Normal Range on Metabolic Function and Body Composition" study. For the purpose of this sub-analysis, data from euthyroid women between 20-74 years of age with either no thyroid disease (controls; n=16) or who received L-T4 treatment for hypothyroidism (n=49) were included. At the time of data collection, all subjects had serum TSH concentrations between 0.34-5.60 mU/L. Body composition, total energy expenditure (TEE), resting energy expenditure (REE), and thermic effect of food (TEF) were measured and macronutrient oxidation rates and respiratory quotient (RQ) were calculated in each participant. All procedures took place at the Oregon Health & Science University (OHSU) Marquam Hill Campus in the Oregon Clinical & Translational Research Institute (OCTRI), in the Clinical Translational & Research Center (CTRC). This study was reviewed and approved by the OHSU Institutional Review Board (IRB) and all participants signed consent and Health Insurance Portability and Accountability Act (HIPAA) forms.

Study Participants

Women treated with L-T4 were hypothyroid as a result of adult-onset disease due to radioactive iodine ablation or thyroidectomy for benign thyroid disease, or Hashimoto's disease. L-T4 treated euthyroid participants had proven elevated serum TSH concentrations before L-T4 treatment or while taking subtherapeutic doses of L-T4. Participants received stable L-T4 doses for at least 3 months with documented serum TSH concentrations in the normal range of 0.34 -5.60 mU/L before participating in study measurements. Participants

did not have any other acute or chronic illnesses that could affect thyroid function or metabolism, and were not taking medications that could affect thyroid hormone concentrations or metabolism. Oral contraceptives and estrogen replacement therapy were allowed, as long as the type and dose were stable for 3 months and no changes were anticipated during the study. Women were studied in the follicular phase of the menstrual cycle or in the first week of an oral contraceptive or estrogen replacement therapy cycle. Perimenopausal women were included in the parent study and in this sub-analysis since they are among the most common patients with hypothyroidism.

Study Visit

Subjects were free-living and completed one study visit and a screening visit to determine eligibility. Visit details are outlined in Table 1.

Anthropometric Measurements

Weight while dressed in a hospital gown, only, was measured and recorded with a digital scale (Scale-Tronix, Model 5002, Wheaton, IL) to the nearest 0.01 kg. Height was measured without shoes using a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Ltd, Crymych, UK). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters-squared.

Table 1. Screening and Baseline Visit Procedures

Screening and Baseline Visit Procedures	Screening	Baseline
Medical History and Physical Exam	X	
Blood Hemoglobin and Glucose Concentrations	X	
Questionnaires	X	X
Urine (HG) Pregnancy test *	X	X
Vital Signs	X	X
Blood Thyroid Hormone Concentrations	X	X
Body Composition (DEXA scan)		X
Resting Energy Expenditure (Indirect Calorimetry)		X
Thermic Effect of Food (Indirect Calorimetry)		X
Total Energy Expenditure (Doubly Labeled Water)		X
24-Hour Urine Sample for Urine Urea Nitrogen		X

* A pregnancy test indicating non-pregnant status was required before completing the DEXA scan.

Measurement of Body Composition by Dual Energy X-ray Absorptiometry

Body composition (lean mass, fat mass, and bone mass) was measured by Dual Energy X-ray Absorptiometry (DEXA) using a Hologic QDR Discovery A Densitometer (Hologic, Inc., Bedford, MA) following standard procedures (31). Study participants were asked to lie horizontally on the DEXA scanning bed, while wearing a hospital gown and after

removing all metal jewelry. The DEXA technician positioned the subject within the appropriate quadrants of the scanning bed and a total-body scan was performed. For women who did not fit completely within the DEXA scanning plane, a hemi-scan was performed and measurements were multiplied by two to determine each body composition component.

Measurement of Total Energy Expenditure by Doubly Labeled Water

Total energy expenditure (TEE) was measured by the doubly labeled water (DLW) method. Participants consumed water enriched with stable isotopes for hydrogen (deuterium ^{2}H) and oxygen (^{18}O). The ^{18}O sample was purchased from Cambridge Isotope Laboratories (Andover, MA) and the ^{2}H sample was purchased from Sigma Aldrich (St. Louis, MO). Each subject drank a premixed dose of water that provided 1.7 gm $^{2}H_{2}{}^{18}O$ per kg of body weight. The dose provided 1.6 g/kg of 94% $^{2}H_{2}{}^{18}O$ and 0.10 g/kg of 99.9% $^{2}H_{2}O$. Spot urine samples were collected before and 2, 3 and 4 hours after consuming the enriched water to determine background exposure and whole body equilibrium, respectively. Two additional spot urine samples were collected 7 days later; upon waking and 1 hour later, to calculate the elimination rates of each stable isotope. Urine samples were stored at -20° C until sent for analysis. During the 7-day interval, ^{2}H was eliminated as water (H_2O) and ^{18}O was eliminated as H_2O and CO_2. The difference between the two isotope elimination rates was equal to the amount of CO_2 produced. The amount of CO_2 produced was used to estimate total energy expenditure. The DLW method allowed subjects to maintain their free living activities and has an accuracy of ~2% and a precision of 3% to 7% (compared to near-continuous respiratory gas exchange, depending on the isotope dose of the elimination period) (32). The within subject coefficient of variation (CV) for enriched samples for $^{2}H^{18}O$ is 0.2% and for $^{2}H_{2}O$ is 2%. The within person variation for total energy expenditure is 7.8% (33).

The ratio of $^{2}H/^{1}H$ in hydrogen gas and $^{18}O/^{16}O$ in carbon dioxide gas was measured using a Europa 20/20 Isotope Ratio Mass Spectrometer in the laboratory of Dale Scholler, PhD, at the University of Wisconsin. Carbon dioxide production was calculated by the equation of Schoeller (34):

$$VCO_2 \text{ (mol/d)} = (TBW/2.076)(1.007k18-1.041/k2)-0.0253rG$$

where TBW is total body water and k_o and k_d represent isotope constant elimination rates calculated by the linear regression of the isotope enrichment over time. rG is a correction factor for the fractionated water loss with breathing and sweating over time.

$$rG=1.05*TBW*(1.007k18-1.041k2)$$

Carbon dioxide production (VCO_2) was used to calculate total energy expenditure by the modified Weir equation (32):

$$TEE= VCO_2 (1.1+3.9/FQ)*22.4L/mol\ CO_2$$

where FQ is the food quotient. The FQ is the ideal diet-specific ratio of VCO_2 to VO_2 using the following equation:

$$FQ= (0.835 \times \%protein) + (1.0 \times \%CHO) + (0.71 \times \%fat) \quad (35)$$

Under conditions of perfect energy and nutrient balance FQ and RQ should be equal (36). For this analysis a constant of 0.86 was used to estimate the food quotient.

Measurement of Resting Energy Expenditure by Indirect Calorimetry

Resting Energy Expenditure (REE) was measured by indirect calorimetry in a thermo-neutral room maintained at 21.1° F. REE was calculated using measurements of the volume of oxygen consumed (VO_2) and the volume of carbon dioxide produced (VCO_2) in

inspired and expired air, respectively. A VMax Encore 29N Indirect Calorimeter (SensorMedics Viasys Healthcare, Yorba Linda, CA) was used to measure VO_2 and VCO_2. This procedure was conducted after the participant fasted for 12 hours and before she performed any significant physical activity. Immediately before the procedure, each subject rested comfortably on a bed next to the indirect calorimeter for 20 minutes. A clear Plexiglas™ canopy was fitted over her head and upper chest to ensure that air-exchange occurred only through the air intake and output valves. Airflow through these valves was adjusted to accommodate the participant. Expired air was sampled and analyzed for the volume of oxygen consumed (VO_2) and the volume of carbon dioxide produced (VCO_2) each minute for 60 minutes. Resting Energy Expenditure (REE) was calculated using the modified Weir equation:

$$REE\ (kcal/d) = [3.941\ (VO_2, L/min) + 1.106\ (VCO_2, L/min)] + [2.17\ (UUN, g/d) \times 1440\ min/day]$$

Respiratory Quotient (RQ) was calculated as the ratio of VCO_2 (L/min) to VO_2 (L/min):

$$RQ = VCO_2\ (L/min)/VO_2\ (L/min)$$

Substrate oxidation rates were calculated using the following equations:

Carbohydrate Oxidation (g/day) =

$$4.59\ (VCO_2, L/min) - 3.25\ (VO_2, L/min - 3.68\ (UUN, g/min)^* 1440\ min/day$$

Fat Oxidation (g/day) =

$$1.69\ (VO_2, L/min) - 1.69\ (VCO_2, L/min) - 1.72\ (UUN, g/min) * 1440\ min/day$$

Protein Oxidation (g/day) =

$$6.25\ g\ Protein / g\ Nitrogen\ (UUN, g/min)^*1440\ min/d$$

where UUN is the concentration of urea nitrogen in a sample of urine obtained from a 24-hour collection. Measurement of UUN is described below in the biochemical analysis section.

Measurement of the Thermic Effect of Food

Thermic effect of food (TEF) was determined by indirect calorimetry immediately after REE was measured. Each participant consumed a standard liquid meal (Ensure, Ross Laboratories) that provided an energy intake of 35% of their REE as measured by indirect calorimetry. The macronutrient composition of the meal was 14% protein, 31.5% fat, and 54.5% carbohydrate. Post-prandial energy expenditure was measured for 15 minutes every half-hour for 5 to 6 hours using the procedure described for REE. For each 15 minute interval, the difference of TEF minus the REE measurement was calculated. Each TEF value was then averaged with the previous measurement. The average TEF value was then divided by 48 (number of 30 minute intervals in a 24 hour period) and the sum of the 10 TEF values was calculated to reflect the TEF associated with consuming a standardized breakfast meal. TEF was then multiplied by 3.5; a constant that represents the typical consumption of three meals and one snack to estimate total TEF within a 24 hour period.

Biochemical Methods

Serum TSH, free thyroxine (FT4) and free triiodothyronine (FT3) concentrations were measured by the Northwest Kaiser Permanente Laboratory in Portland, OR.

Thyroid Stimulating Hormone

Thyroid Stimulating Hormone (TSH) was measured in mU/L by an immunochemiluminometric assay (ICMA, Kaiser Permanente NW Regional Lab, Beckman

Coulter counter™, Portland OR). The analytic sensitivity of the assay is 0.003 mU/L, the functional sensitivity is 0.02 mU/L, and the normal range is 0.34–5.60 mU/L. The intra-assay CV is 9.5% at 0.03 mU/L and 4.7% at 11.6 mU/L, and the inter-assay CV is 11% at 0.04 mU/L, 5% at 0.70 mU/L, and 5.8 % at 24.94 mU/L (37).

Free Thyroxine (FT4)

Free thyroxine (FT4) was measured by an immunochemiluminometric assay (Quest Diagnostic, Nichols Diagnostic Institute Kit 35167X, San Juan Capistrano, CA). The sensitivity of this assay is 0.08 ng/dL. The intra-assay CV is concentration dependent: the intra-assay CV at a lower concentration is 0.27ng/dL (5.7%) and at a higher concentration is 4.6 ng/dL (1%). The inter-assay CV is 0.3 ng/dL (6.8%) and 3.8 ng/dL (1.6%) (38).

Free Triiodothyronine (FT3)

Free triiodothyronine (FT3) was measured by tracer dialysis (Quest Diagnostic, Nichols Diagnostic Institute Kit 36598X, San Juan Capistrano, CA). The sensitivity of this assay is 25 pg/dL. The intra-assay CV is 6% and the inter-assay CV is 4% (38).

24- Hour Urine Collection and Measurement of Urine Urea Nitrogen Concentration

Participants collected urine for a 24-hour period prior to their first baseline clinic visit. All urine was collected, mixed, and the total volume recorded. An aliquot of the total urine sample was obtained and stored frozen pending analysis. Urine urea nitrogen (UUN) concentration was measured at the Clinical and Translational Research Center Core Laboratory using an autoanalyzer (Beckman Coulter counter™) by the following reactions: urea was hydrolyzed to ammonia and CO_2 by urease. Then glutamate dehydrogenase was added to catalyze the condensation of ammonia and alpha-ketoglutarate to form glutamate and nicotinamide adenine dinucleotide (NAD). In this process, the hydrogenated form of

nicotinamide adenine dinucleotide (NADH) is converted to NAD. The conversion of NADH to NAD results in an absorbance reading measured at 340 nm which is directly related to UUN concentrations. UUN concentration was recorded in mg/dL based on the change in absorbance calculated from point slope calculations. Nitrogen excretion was calculated by multiplying urea concentration (g/dL) by a conversion factor of 0.46 (mg/dL) nitrogen. Nitrogen excretion was converted to a 24 hour excretion rate (g/day) by dividing nitrogen (g/mL) by the total volume of urine excreted in 24 hours (mL/24 hours). UUN was converted to a rate of g/min by dividing total nitrogen excreted in 24 hours (g/ 24 hours) by 1440 minutes/24 hours.

Data Cleaning and Evaluation

Data collected from total energy expenditure, resting energy expenditure, thermic effect of food, macronutrient oxidation of fat, carbohydrate and protein, and measures of body composition was transferred into standard spreadsheets (Excel, Microsoft Office 2010 and Statistical Package for Social Sciences (SPSS). Standard distribution curves were generated to assess normality of each set of outcome variables. Box-plots were used to identify outliers and skewedness. Data points that stood out from the others by visual inspection were investigated further to ensure data was entered correctly. Participants were excluded from this analysis if they had incomplete data sets.

Statistical Analysis

Subjects (n= 65) were divided into two groups: those with low-normal TSH concentrations between 0.34 – 2.49 mU/L and those with high-normal TSH concentrations between 2.50 – 5.60 mU/L. Descriptive statistics including mean, standard deviation, minimum and maximum values were calculated for each outcome variable by group and for the entire sample. Two sets of analyses were conducted: serum TSH concentrations were treated both as a continuous variable and as a categorical variable (low-normal versus high-normal). Independent sample t-tests were used to compare mean differences between the low-normal and high-normal TSH groups followed by regression analyses to adjust for potential confounders. P-values less than 0.05 were considered significant. Linear regression was also used to investigate the association between serum TSH concentrations and total energy expenditure, resting energy expenditure, thermic effect of food, macronutrient oxidation rates, and measures of body composition including total mass, lean mass, fat mass and percent body fat. Each relationship between TSH concentration and outcome variables was adjusted individually for age, BMI, race (White vs. non-White), menstrual stage (1. premenopausal, no hormonal contraception 2. premenopausal, on hormone contraception, 3. post menopausal, no hormonal contraception, and 4. postmenopausal, on hormonal contraception), L-T4 treatment, and adjustment for all of these factors together. The magnitude and direction of the mean differences, 95% confidence intervals, and p-values were obtained for all models. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) was used for all analyses.

Chapter 4: Results

Descriptive Statistics

A cross-sectional study of 65 women with serum TSH concentrations within the established normal range of 0.34 – 5.60 mU/L was performed to determine if energy expenditure, macronutrient oxidation rates, and body composition variables were different among individuals with low-normal TSH concentrations (0.34 – 2.49 mU/L; n = 46) compared to those with high-normal TSH concentrations (2.50 – 5.60 mU/L; n = 19). The distribution of participants into low-normal (n = 46) and high-normal (n= 19) TSH concentration groups and control, no L-T4 treatment (n=16) and L-T4 treated groups (n=49) is illustrated in Figure 3. Of the 65 participants, 5 were missing serum FT4 and FT3 concentration measurements, but were still included in the final analysis because their TSH values were available.

Biochemical, demographic, and anthropometric characteristics of the study participants are presented in Table 2. The average (± SD) serum TSH concentration was 2.04 ± 1.18 mU/L for women in both groups, combined. Seventy-five percent of the participants were treated with L-T4 for hypothyroidism and of those, the average (± SD) treatment dose was 1.46 ± 0.51 mcg/kg body weight. The average (± SD) age was 44.8 ± 11.4 years, 84.6% were white, non-Hispanic and 41.5% were premenopausal and did not use hormonal contraception. The average (± SD) weight and BMI was 75.5 ± 18.0 kg and 28.1 ± 6.4 kg/m^2, respectively.

When participants were grouped by serum TSH concentrations, the L-T4 treatment dose, body weight and BMI were different between groups ($p = 0.05$). The average TSH concentration of the low-normal TSH concentration group was 1.42 ± 0.65 mU/L compared to 3.54 ± 0.76 mU/L in the high-normal TSH concentration group. Eighty percent of participants were treated with L-T4 in the low-normal group compared to 63.2% in the high-

normal group. The average L-T4 treatment dose of 1.52 ± 0.51 mcg/kg in the low-normal TSH group was significantly higher than the 1.26 ± 0.46 mcg/kg L-T4 treatment dose in the high-normal TSH group (p=0.041). The average weight (72.7 ± 15.3 kg) in the low-normal TSH concentration group was significantly lower than the high-normal TSH group (82.2 ± 22.3 kg, p=0.05). The difference in mean weight was 9.5 kg (95% CI: -0.10 – 19.1 kg). Likewise, the low-normal TSH group had a significantly lower average BMI (27.1 ± 5.4 kg/m^2) than the high-normal TSH group (30.5 ± 8.0 kg/m^2, p=0.05). The difference in mean BMI between the two groups was 3.4 kg/m^2 (95% CI: 0.01 - 6.86 kg/m^2).

Table 3 presents a summary of the biochemical, demographic, and anthropometric characteristics of the study participants when grouped by control and L-T4 treatment status. There were no statistically significant differences between the two groups in any of these variables.

Body composition variables assessed by DEXA among women categorized by low and high-normal serum TSH concentrations are presented in Table 4. Total mass is the sum of lean mass, fat mass and bone mass and is slightly different than total body weight measured on calibrated digital scale. There were no significant differences in mean total body mass, lean mass, fat mass and percent body fat between groups. Although not statistically significant, the mean difference in percent body fat between groups was 3.8% (95% CI: -0.55 – 8.11 %, p-value =0.09) which approached significance.

The average total energy expenditure, resting energy expenditure, and the thermic effect of food of the low- and high-normal TSH concentration groups are presented in Table 5. There were no significant differences in any energy expenditure variables between the two groups, even when energy expenditure was corrected for total body mass and lean mass. The only difference that approached significance was in total energy expenditure where the two groups differed by an average of 208 kcals/d (95% CI: -29.3 – 445, p-value=0.09).

Fasting respiratory quotient (RQ) and macronutrient oxidation rates for the low- and high-normal TSH concentrations groups are presented in Table 6. There were no significant differences in average measures of RQ or macronutrient oxidation rates between the two groups.

Linear Regression Models with Serum TSH Concentration Dichotomized as Low-Normal or High-Normal

The unadjusted and adjusted linear regression models for body composition, energy expenditure, and macronutrient oxidation rates versus serum TSH concentrations categorized into the low-normal TSH group and high-normal TSH group are presented in Table 7. The only significant relationships observed were in weight ($p = 0.05$) and in BMI ($p = 0.05$) when plotted against serum TSH concentration. The relationship of serum TSH concentrations and BMI remained significant when adjusted for age ($p = 0.05$) and race ($p = 0.048$), however was less significant when adjusted for the L-T4 treatment group ($p = 0.06$), estrogen status ($p = 0.06$) and all other factors combined ($p = 0.07$). All other outcome variables (e.g. weight, total body mass, total energy expenditure, etc.) showed no significant relationships when plotted against low-normal and high-normal serum TSH concentrations. The relationship between serum TSH and percent body fat approached significance when unadjusted ($p = 0.09$) and adjusted for age ($p = 0.08$), race ($p = 0.09$), and estrogen status ($p = 0.06$).

Linear Regression Models with Serum TSH Concentration as a Continuous Variable

The unadjusted and adjusted linear regression models for body composition, energy expenditure, and macronutrient oxidation rates in relation to serum TSH concentrations are presented in Table 8. When each outcome variable (e.g. weight, BMI, total body mass, etc.) was regressed against serum TSH concentration, no significant linear relationships were

observed. Likewise when the relationships between outcome variables and serum TSH concentrations were adjusted individually, as well as simultaneously, for age, BMI, race, L-T4 treatment group, estrogen status, there were no significant differences between those relationships with serum TSH. However, the relationship between weight and serum TSH concentration approached significance ($p = 0.08$) when weight was adjusted for estrogen status. The linear relationship between TEE and serum TSH concentration approached significance ($p = 0.07$). Likewise, when adjusted for race and L-T4 treatment group, the relationship between TEE and serum TSH concentration approached significance ($p = 0.07$ and $p = 0.09$, respectively).The relationship between serum TSH concentration and TEE/kg body weight (kcal/kg) approached significance when adjusted for BMI ($p = 0.08$). No trends were identified when macronutrient oxidation rates, in relation to serum TSH concentration, were adjusted for age, BMI, race, L-T4 treatment group, estrogen status, or when all factors were considered together.

Considering our initial hypotheses that:

1. Energy expenditure and fat oxidation rates will be higher among women with serum TSH concentrations within the low-normal TSH group.
2. Body fat percentage and protein and carbohydrate oxidations will be higher among women in the high-normal TSH group

and the information presented here, we reject our hypotheses that energy expenditure and fat oxidation rates are higher in women within the low-normal TSH range (0.34 - 2.49 mU/L) and body fat percentage and protein and carbohydrate oxidation rates are higher among women with serum TSH concentrations within the high-normal range (2.50 – 5.60 mU/L).

Figure 3. Distribution of Participants into Low-Normal and High-Normal Serum TSH Concentration Groups and Healthy Control and Levothyroxine (L-T4) Treated Groups.

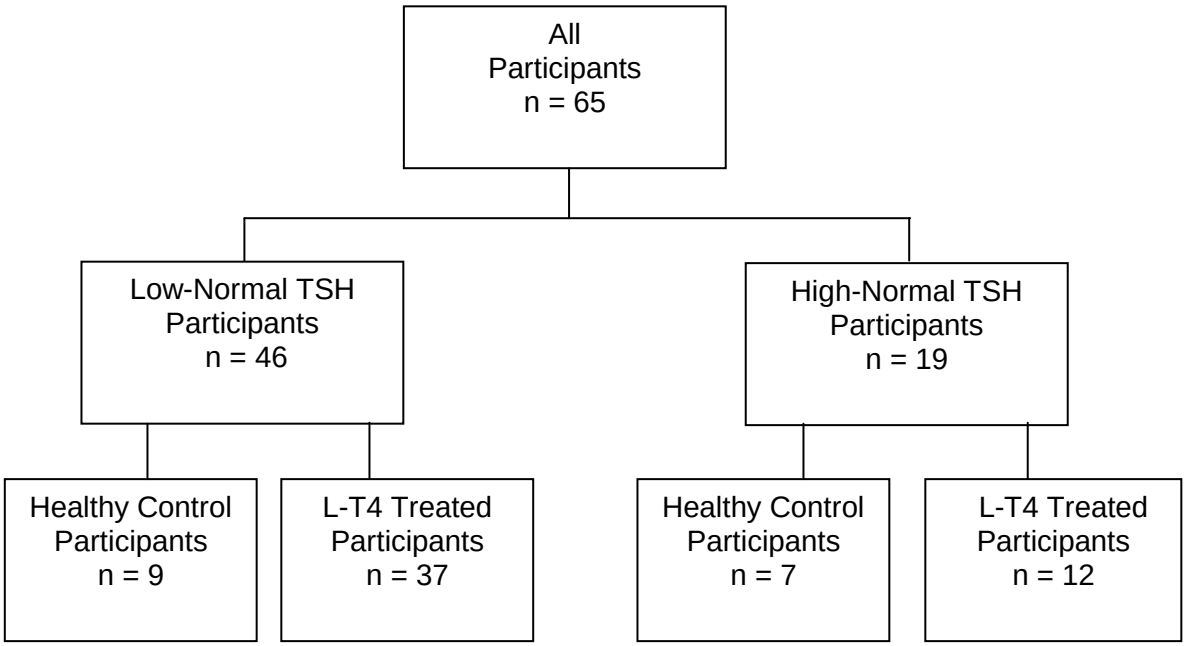

Table 2. Biochemical, Demographic, and Anthropometric Characteristics of Women in Low-Normal or High-Normal Serum TSH Concentration Groups*			
Biochemical Variables	**All Participants (n=65)**	**Low-Normal TSH (n=46)**	**High-Normal TSH (n=19)**
Thyroid Stimulating Hormone (mU/L)	2.04 ± 1.18 (0.34 – 4.87)	1.42 ± 0.65 (0.34 – 2.46)	3.54 ± 0.76 (2.60 – 4.87)
L-T4 Treated (%)	75.4	80.4	63.2**
L-T4 Treatment Dose (mcg/kg)°	1.46 ± 0.51 (0.60 – 3.03)	1.52 ± 0.51 (0.60 – 3.03)	1.26 ± 0.46** (0.73 – 2.21)
FT4 (ng/dL)	1.55 ± 0.40[a] (1.00 – 2.90)	1.62 ± 0.42[b] (1.00 - 2.90)	1.36 ± 0.25[c] (1.00 – 1.90)
FT3 (pmol/L)	223 ± 36.4 [a] (160 - 316)	223 ± 35.7[b] (170 - 316)	221 ± 39.0[c] (160 – 301)
Demographic Characteristics			
Age (yr)	44.8 ± 11.4 (20.6 – 68.0)	45.0 ± 11.5 (20.6 – 68.0)	44.2 ± 11.4 (28.4 – 67.4)
Race (White, non-Hispanic, %)	84.6	86.2	98.5
Estrogen Status (%)			
Premenopausal, no hormonal contraception	41.5	39.1	47.4
Premenopausal, on hormonal contraception	24.6	23.9	26.3
Postmenopausal, no hormonal contraception	27.7	30.4	21.1
Postmenopausal, on hormonal contraception	6.2	6.5	5.3
Anthropometric Characteristics			
Height (cm)	164 ± 6.4 (149 – 180)	164 ± 6.9 (149 – 180)	164 ± 4.3 (152 – 171)
Weight (kg)	75.5 ± 18.0 (48.6 – 138)	72.7 ± 15.3 (48.6 – 105)	82.2 ± 22.3** (56.3 -138)
Body Mass Index (kg/m^2)	28.1 ± 6.4 (19.1 – 47.4)	27.1 ± 5.4 (19.1 – 42.7)	30.5 ± 8** (20.4 – 47.4)
Mean ± SD (range)* **p-value <0.05 compared to low-normal TSH group using independent t-tests °In L-T4 treated participants only, [a] n=60, [b] n=43, [c] n=17			

Table 3. Biochemical, Demographic, and Anthropometric Characteristics of Women in the Control (C) vs the Levothyroxine (L-T4) Treated Groups *		
Biochemical Variables	**Control Group (n=16)**	**L-T4 Treated Group (n=49)**
Thyroid Stimulating Hormone (mU/L)	2.2 ± 1.0 (0.9 – 4.0)	2.0 ± 1.2 (0.34 – 4.9)
FT4 (ng/dL)	1.3 ± 0.2[a] (1.0 – 1.7)	1.6 ± 0.4[b] (1.0 – 2.9)
FT3 (pmol/L)	236 ± 42.6[a] (178 – 301)	218 ± 33.3[b] (160 – 316)
Demographic Characteristics		
Age (yr)	42.5 ± 11.2 (20.6 – 56.9)	45.6 ± 11.4 (27.3 – 68.0)
Race (White, non-Hispanic, %)	90.0	90.0
Estrogen Status (%)		
Premenopausal, no hormonal contraception	37.5	42.9
Premenopausal, on hormonal contraception	31.3	22.4
Postmenopausal, no hormonal contraception	25.0	28.6
Postmenopausal, on hormonal contraception	6.2	6.1
Anthropometric Characteristics		
Height (cm)	165 ± 7.0 (154 – 180)	164 ± 6.0 (149 – 177)
Weight (kg)	78.4 ± 22.0 (51.3 -117)	74.6 ± 16.6 (48.6 – 138)
Body Mass Index (kg/m^2)	28.9 ± 7.6 (21.1 – 44.5)	27.8 ± 6.1 (19.1 – 47.4)
*Mean ± SD (range) [a] n=15, [b] n= 45		

Table 4. Body Composition Variables Among Women in the Low-Normal or High-Normal Serum TSH Concentration Groups*			
Body Composition Variables	**All Participants (n=65)**	**Low-Normal TSH Group (n= 46)**	**High-Normal TSH Group (n= 19)**
Total Body Mass (kg)	74.9 ± 18.2 (47.5 – 137)	72.6 ± 15.4 (47.5 – 106)	80.3 ± 23.3 (51.1 – 137)
Lean Mass (kg)	45.7 ± 6.5 (34.1 -67.3)	45.0 ± 6.1 (34.1 - 59.2)	47.4 ± 7.4 (39.9 – 67.3)
Fat Mass (kg)	27.1 ± 12.2 (10.3 – 67.0)	24.9 ± 9.9 (10.3 – 46.2)	32.4 ± 15.6 (14.0 – 67.0)
Percent Body Fat (%)	34.9 ± 8.1 (19.9 – 52.2)	33.8 ± 7.6 (19.9 – 48.8)	37.6 ± 8.7 (22.7 – 52.2)
*Mean ± SD (range)			

Table 5. Energy Expenditure Variables of Women in the Low-Normal or High-Normal Serum TSH Concentration Groups*			
	All Participants (n=65)	**Low – Normal TSH Group (n = 46)**	**High – Normal TSH Group (n = 19)**
Energy Expenditure Variables			
Total Energy Expenditure (kcal/d)	2336 ± 442 (1561 – 3354)	2275 ± 413 (1561 – 3264)	2483 ± 485 (1698 – 3354)
(kcal/kg body weight)	32.0 ± 7.2 (21.3 – 49.3)	32.1 ± 6.6 (22.7 – 48.6)	31.6 ± 8.7 (21.3 – 49.3)
(kcal/kg lean mass)	51.4 ± 8.6 (35.7-77.4)	50.7 ± 7.6 (35.7 – 71.2)	52.9 ± 10.7 (42.0 – 77.4)
Resting Energy Expenditure (kcal/d)	1290 ± 183 (1039 -1798)	1269 ± 162 (1039 – 1662)	1340 ± 223 (1060 – 1798)
(kcal/kg body weight)	17.8 ± 3.3 (13.0 – 31.0)	17.9 ± 2.9 (13.7 – 24.8)	17.5 ± 4.2 (13.0 – 31.0)
(kcal/kg lean mass)	28.3 ± 2.6 (23.6 – 34.5)	28.4 ± 2.8 (23.6 – 34.5)	28.3 ± 2.1 (24.2 – 32.4)
Resting Energy Expenditure/Total Energy Expenditure (%)	56.2 ± 7.5 (35.5 – 69.4)	56.7 ± 6.9 (38.9 – 69.4)	55.0 ± 8.9 (35.5 – 67.6)
Thermic Effect of Food (kcal/meal)	41.1 ± 15.3 (8.3 – 86.2)	40.3 ± 15.1 (8.3 – 76.9)	43.1 ± 16.0 (22.6 – 86.2)
Thermic Effect of Food (kcal/day)[a]	144 ± 53.6 (29.1 – 302)	141 ± 52.8 (29.1 – 269)	151 ± 56.1 (79.1 – 302)
Thermic Effect of Food/Total Energy Expenditure (%)[b]	6.3 ± 2.3 (1.3 – 11.8)	6.3 ± 2.4 (1.3 – 11.8)	6.1 ± 2.0 (3.2 – 10.0)

* Mean ± SD (range)

[a] Thermic Effect of Food (kcal/d) was calculated by multiplying TEF (kcal/meal) by a factor of 3.5 representing 3 meals and a snack

[b] TEF/TEE (%) is TEF in kcal/d divided by TEE kcal/d

Table 6. Macronutrient Oxidation Variables Among Women in the Low-Normal or High-Normal Serum TSH Concentration Group*			
Macronutrient Oxidation Variables	**All Participants (n=65)**	**Low – Normal TSH Group (n = 46)**	**High – Normal TSH Group (n = 19)**
Respiratory Quotient	0.84 ± 0.05 (0.76 – 0.96)	0.84 ± 0.05 (0.76 – 0.96)	0.83 ± 0.05 (0.76 – 0.91)
Carbohydrate Oxidation Rate (g/d)	127 ± 57.8 (20.0 – 269)	128 ± 61.3 (20.0 – 269)	123 ± 49.6 (24.8 – 208)
Fat Oxidation Rate (g/d)	58.5 ± 27.0 (0.7 – 118)	55.8 ± 24.6 (0.7 – 118)	64.9 ± 31.9 (21.5 – 117)
Protein Oxidation Rate (g/d)	52.7 ± 22.4 (4.7 – 131)	53.0 ± 24.1 (4.7 – 131)	52.2 ± 18.1 (12.0 – 87.6)
Total Energy Oxidized (kcals/d)**	1243 ± 174 (994 – 1720)	1226 ± 157 (994 – 1561)	1286 ± 210 (1014 – 1720)
Total Energy Oxidized (kcals/d)/Resting Energy Expended (kcal/d), (%kcal/REE)	96.5 ± 3.2 (88.6 – 112)	96.6 ± 3.6 (88.6 – 112)	96.1 ± 1.9 (91.3 – 99.1)
Total Energy Oxidized (kcals/d)/Total Energy Expended (kcal/d), (%kcal/TEE)	34.0 ± 10.0 (15.9 – 57.2)	35.0 ± 10.3 (15.9 – 57.2)	31.8 ± 9.3 (17.0 – 48.8)
*Mean ± SD (range) ** Total Energy Oxidized = Grams Carbohydrate Oxidized (*4 kcal/g) + Grams Fat Oxidized (*9 kcal/g) + Grams Protein Oxidized (*4 kcal/g)			

Table 7. The linear relationship between body composition, energy expenditure and macronutrient oxidation rates and low-normal and high-normal serum TSH concentrations.

	Weight (kg)	Body Mass Index (kg/m^2)	Total Body Mass (kg)	Lean Body Mass (kg)	Fat Mass (kg)	Percent Body Fat (%)
Serum TSH Concentration	-9.49 [-19.1 – 0.1] (0.05)**	-3.43 [-6.86 - 0.00] (0.05)**	-7.61 [-17.4 - 2.17] (0.13)	-2.43 [-5.97 - 1.11] (0.18)	-4.32 [-10.5 - 1.85] (0.17)	-3.78 [-8.12 - 0.55] (0.09)
Adjusted for: Age	-9.44 [-19.1 – 0.23] (0.06)	-3.41 [-6.87 - 0.05) (0.05)**	-7.58 [-17.4 - 2.29] (0.13)	-2.37 [-5.92 - 1.17] (0.19)	-4.35 [-10.6 - 1.89] (0.17)	-3.86 [-8.19 -0.48] (0.08)
BMI	-0.47 [-3.89 – 2.94] (0.78)		1.32 [-2.83 - 5.47] (0.53)	0.15 [-2.37 - 2.67] (0.90)	0.17 [-4.24 - 4.58] (0.94)	-0.07 [-2.39 - 2.26] (0.95)
Race	-8.90 [-18.7 – 0.85] (0.07)	-3.53 [-7.03 – (-0.03)] (0.048)**	-7.08 [-17.0 - 2.88] (0.16)	-2.03 [-5.59 - 1.53] (0.26)	-4.35 [-10.7 - 1.97] (0.17)	-3.29 [-8.25 - 0.60] (0.09)
L-T4 Treatment	-9.13 [-18.9 – 0.70] (0.07)	-3.36 [-6.81 - 0.16] (0.06)	-7.05 [-17.1 - 2.94] (0.16)	-2.42 [-6.05 - 1.21] (0.19)	-3.56 [-9.81 - 2.69] (0.26)	-3.63 [-8.07 - 0.81] (0.11)
Estrogen Status	-9.36 [-19.0 – 0.29] (0.06)	-3.34 [-6.78 - 0.10] (0.06)	-7.55 [-17.5 - 2.36 (0.13)	-2.21 [-5.80 - 1.37] (0.22)	-4.34 [-10.2 - 1.57] (0.15)	-4.07 [-8.16 - 0.13] (0.06)
All factors considered together	0.56 [-2.71 – 3.83] (0.73)	-3.37 [-7.00 - 0.27] (0.07)	2.47 [-1.74 - 6.77] (0.24)	0.92 [-1.52 - 3.36] (0.45)	0.43 [-3.94 - 4.79] (0.85)	-0.48 [-2.69 - 1.72] (0.66)

*Mean Difference
[95% CI]
(p-value)
**p-value is <0.05 when the relationship between TSH and energy expenditure, macronutrient oxidation and body composition is measured using linear regression

Table 7 continued; The linear relationship between body composition, energy expenditure and macronutrient oxidation rates and low-normal and high-normal serum TSH concentrations.

	TEE (kcal/d)	TEE (kcal/kg)	TEE (kcal/kg LBM)	REE (kcal/d)	REE (kcal/kg)	REE (kcal/kg LBM)	TEF (kcal/meal)
Serum TSH Concentration	-208 [-445 - 29.4] (0.09)	0.46 [-3.47 -4.40] (0.81)	-2.13 [-6.82 - 2.56] (0.37)	-70.5 [-170 - 28.5] (0.16)	0.47 [-1.33 -2.28] (0.60)	0.12 [-1.32 - 1.58] (0.87)	-2.85 [-11.2 – 5.53] (0.50)
Adjusted for: Age	-202 [-437 - 32.9] (0.09)	0.56 [-3.35 - 4.46] (0.78)	-2.05 [-6.75 - 2.64] (0.39)	-66.7 [-162 - 28.6] (0.17)	0.53 [-1.22 -2.29] (0.55)	0.17 [-1.22 - 1.56] (0.81)	-2.67 [-11.2 - 5.67] (0.51)
BMI	-132 [-366 – 102] (0.26)	-2.02 [-5.19 - 1.16] (0.21)	-0.32 [-8.04 - 1.37] (0.16)	-2.77 [-77.9 - 72.3] (0.94)	-0.60 [-2.11 -0.90] (0.43)	-0.02 [-1.51 - 1.47] (0.98)	-0.19 [-8.45 – 8.07] (0.96)
Race	-195 [-436 - 46.8] (0.11)	0.32 [-3.40 - 4.33] (0.88)	-2.33 [-7.11 - 2.45] (0.33)	-70.7 [-172 - 30.4] (0.17)	0.32 [-1.51 -2.15] (0.73)	-0.15 [-1.58 - 1.26] (0.84)	-1.25 [-9.42 - 6.92] (0.76)
L-T4 Treatment	-178 [-417 - 61.9] (0.14)	0.77 [-3.24 - 4.78] (0.70)	-1.47 [-6.20 - 3.25] (0.54)	-58.8 [-159 - 41.6] (0.25)	0.52 [-1.33 -2.38] (0.58)	0.37 [-1.06 - 1.81] (0.61)	-3.32 [-3.09 - 5.81] (0.54)
Estrogen Status	-187 [-424 - 51.3] (0.12)	0.76 [-2.98 - 4.49] (0.69)	-1.86 [-6.56 - 2.84] (0.43)	-60.4 [-159 - 38.4] (0.23)	0.62 [-1.11 -2.35] (0.48)	0.22 [-1.18 - 1.62] (0.75)	-2.29 [-11.9 – 5.25] (0.44)
All factors considered together	-53.1 [-292 – 186] (0.66)	-1.31 [-4.47 - 1.85] (0.41)	-2.44 [-7.37 - 2.48] (0.33)	23.9 [-47.5 - 95.2] (0.51)	-0.48 [-1.98 -1.02] (0.53)	0.10 [-1.35 - 1.55] (0.89)	1.84 [-6.27 – 9.94] (0.65)

*Mean Difference
[95% CI]
(p-value)
**p-value is <0.05 when the relationship between TSH and energy expenditure, macronutrient oxidation and body composition is measured using linear regression

Table 7 continued; The linear relationship between body composition, energy expenditure and macronutrient oxidation rates and low-normal and high-normal serum TSH concentrations.

	Carbohydrate Oxidation (g/d)	Fat Oxidation (g/d)	Protein Oxidation (g/d)
Serum TSH Concentration	4.65 [-27.1 - 36.3] (0.77)	-9.12 [-23.8 - 5.53] (0.22)	0.75 [-11.5 - 13.0] (0.90)
Adjusted for: Age	5.34 [-26.2 - 36.8] (0.74)	-9.02 [-23.8 - 5.73] (0.23)	0.87 [-11.5 - 13.2] (0.89)
BMI	9.59 [-22.9 - 42.2] (0.56)	2.13 [-18.5 - 10.2] (0.56)	0.87 [-11.9 - 13.6] (0.89)
Race	-3.15 [-33.1 - 26.8] (0.83)	-7.05 [-21.6 - 7.54] (0.34)	2.30 [-10.0 - 28.9] (0.71)
L-T4 Treatment	4.24 [-28.3 -36.8] (0.80)	-6.76 [-21.4 - 7.90] (0.36)	-0.79 [-13.2 - 11.6] (0.90)
Estrogen Status	5.03 [-26.2 - 36.3] (0.75)	-8.58 [-22.8 - 5.68] (0.23)	1.53 [-10.9 - 13.9] (0.81)
All factors considered together	0.34 [-30.0 - 30.7] (0.98)	1.35 [-12.3 - 15.0] (0.85)	2.63 [-10.4 - 15.6] (0.69)

*Mean Difference
[95% CI]
(p-value)
** p-value is <0.05 when the relationship between TSH and energy expenditure, macronutrient oxidation and body composition is measured using linear regression

Table 8. The linear relationship between body composition, energy expenditure and macronutrient oxidation rates and serum TSH concentration as continuous variable.						
	Weight (kg)	Body Mass Index (kg/m^2)	Total Body Mass (kg)	Lean Body Mass (kg)	Fat Mass (kg)	Percent Body Fat (%)
Serum TSH Concentration	2.79 [-0.97 – 6.55] (0.14)	0.90 [-0.45 – 2.25] (0.19)	2.25 [-1.56 – 6.07] (0.24)	0.93 [-0.44 – 2.30] (0.18)	-0.24 [-2.45 – 2.40] (0.99)	0.65 [-1.06 – 2.36] (0.45)
Adjusted for: Age	2.74 [-1.11 – 6.60] (0.16)	0.87 [-0.52 - 2.25] (0.22)	2.23 [-1.75 - 6.14] (0.26)	0.82 [-0.57 - 2.22] (0.24)	0.002 [-2.49 - 2.49] (0.91)	0.83 [-0.90 - 2.57] (0.34)
BMI	0.43 [-0.86 – 1.73] (0.51)		-0.07 [-1.65 - 1.52] (0.93)	0.26 [-0.70 - 1.22] (0.59)	-1.23 [-2.88 - 0.42] (0.14)	-0.33 [-1.23 - 0.55] (0.45)
Race	2.68 [-1.09 – 6.45] (0.16)	0.90 [-0.46 - 2.27] (0.19)	2.16 [-1.68 - 5.99] (0.27)	0.86 [-0.50 -2.22] (0.21)	-0.04 [-2.49 - 2.41] (0.975)	0.65 [-1.08 - 2.37] (0.46)
L-T4 Treatment	2.69 [-1.11 – 6.48] (0.16)	0.87 [-0.49 - 2.24] (0.21)	2.12 [-1.72 - 5.96] (0.27)	0.91 [-0.47 - 2.31] (0.19)	-0.19 [-2.60 - 2.22] (0.87)	0.60 [-1.12 - 2.33] (0.49)
Estrogen Status	3.36 [-0.41 – 7.91] (0 .08)	1.06 [-0.31 - 2.44] (0.13)	2.76 [-1.16 - 6.68] (0.17)	1.02 [-0.39 - 2.43] (0.15)	0.59 [-1.77 - 2.95] (0.62)	1.12 [-0.55 - 2.77] (0.19)
All factors considered together	0.12 [-1.14 – 1.37] (0.85)	1.03 [-0.40 - 2.47] (0.15)	-0.41 [-2.05 - 1.23] (0.62)	-0.05 [-0.99 - 0.90] (0.92)	-0.88 [-2.55 - 0.79] (0.30)	0.05 [-0.81 - 0.90] (0.92)
*Slope Estimate [95% CI] (p-value)						

Table 8 continued; The linear relationship between body composition, energy expenditure and macronutrient oxidation rates and serum TSH concentration as a continuous.

	TEE (kcal/d)	TEE (kcal/kg)	TEE (kcal/kg LBM)	REE (kcal/d)	REE (kcal/kg)	REE (kcal/kg LBM)	TEF (kcal/meal)
Serum TSH Concentration	85.9 [-5.52 - 177] (0.07)	0.40 [-1.12 - 1.92] (0.60)	1.03 [-0.78 - 2.84] (0.26)	26.8 [-11.5 - 65.1] (0.17)	0.10 [-0.69 -0.71] (0.98)	0.01 [-0.54 - 0.57] (0.96)	0.69 [-2.56 – 3.94] (0.67)
Adjusted for: Age	75.7 [-16.8 – 168] (0.18)	0.21 [-1.33 - 1.74] (0.79)	0.89 [-0.96 - 2.73] (0.34)	19.1 [-18.6 - 56.8] (0.32)	-0.13 [-0.82 -0.57] (0.72)	-0.10 [-0.65 - 0.45] (0.71)	-0.52 [-2.79 – 3.84] (0.75)
BMI	65.8 [-22.4 - 154] (0.14)	1.05 [-0.15 - 2.24] (0.08)[b]	1.33 [-0.46 - 3.12] (0.14)	9.29 [-19.2 - 37.8] (0.52)	0.29 [-0.28 -0.86] (0.31)	0.05 [-0.51 - 0.62] (0.86)	-0.12 [-3.16 – 3.13] (0.15)
Race	83.5 [-8.53 - 175] (0.07)	0.43 [-1.11 - 1.96] (0.58)	1.06 [-0.77 - 2.88] (0.25)	26.7 [-12.0 - 65.4] (0.17)	0.03 [-0.67 -0.73] (0.92)	0.05 [-0.49 - 0.59] (0.84)	0.44 [-2.68 – 3.57] (0.78)
L-T4 Treatment	79.7 [-11.2 - 171] (0.09)	0.35 [-1.18 - 1.88] (0.65)	0.91 [-0.89 - 2.70] (0.32)	24.4 [-13.8 - 62.6] (0.21)	0.004 [-0.70 -0.71] (0.99)	-0.03 [-0.58 - 0.52] (0.91)	0.76 [-2.51 – 4.04] (0.64)
Estrogen Status	74.8 [-19.0 – 169] (0.12)	-0.07 [-1.55 - 1.41] (0.93)	0.68 [-1.18 - 2.54] (0.47)	21.9 [-17.2 - 60.9] (0.27)	-2.07 [-0.89 -0.48] (0.55)	-0.16 [-0.71 - 0.39] (0.56)	0.93 [-2.39 – 4.26] (0.58)
All factors considered together	36.4 [-55.3 - 128] (0.43)	0.64 [-0.57 - 1.85] (0.30)	1.01 [-0.89 - 2.91] (0.29)	-6.28 [-33.9 - 21.3] (0.65)	0.10 [-0.49 -0.68] (0.74)	-0.11 [-0.67 - 0.45] (0.69)	-0.63 [-3.57 – 2.50] (0.69)

*Slope Estimate
[95% CI]
(p-value)

Table 8 continued; The linear relationship between body composition, energy expenditure and macronutrient oxidation rates and serum TSH concentration as a continuous variable.			
	Carbohydrate Oxidation (g/d)	Fat Oxidation (g/d)	Protein Oxidation (g/d)
Serum TSH Concentration	-3.43 [-15.8 - 8.81] (0.58)	3.33 [-2.34 - 9.00] (0.25)	1.25 [-3.49 - 5.99] (0.60)
Adjusted for: Age	-5.09 [-17.4 - 7.24] (0.41)	3.22 [-2.60 - 9.03] (0.27)	1.02 [-3.83 - 5.86] (0.68)
BMI	-4.71 [-17.1 - 7.66] (0.45)	2.03 [-3.42 - 7.47] (0.46)	1.27 [-3.58 - 6.11] (0.60)
Race	-2.28 [-13.7 - 9.16] (0.69)	3.00 [-2.57 - 8.57] (0.29)	1.03 [-3.68 - 5.73] (0.66)
L-T4 Treatment	-3.36 [-15.8 - 9.04] (0.59)	2.86 [-2.78 - 8.45] (0.31)	1.55 [-3.18 - 6.27] (0.52)
Estrogen Status	-2.70 [15.0 - 9.62] (0.66)	2.65 [-3.02 - 8.31] (0.35)	0.86 [-4.05 - 5.76] (0.73)
All factors considered together	-2.66 [-14.4 - 9.03] (0.65)	0.02 [-5.25 - 5.30] (0.99)	0.26 [-4.75 - 5.28] (0.92)
*Slope Estimate [95% CI] (p-value)			

Chapter 5: Discussion

Summary

This cross-sectional study examined relationships between serum TSH concentrations within the normal reference range and markers of energy expenditure, macronutrient oxidation and body composition. Study participants were healthy control or euthyroid L-T4 treated women with serum TSH concentrations within 0.34 – 4.89 mU/L who were categorized into two groups: low-normal serum TSH (0.34 – 2.49 mU/L) and high-normal serum TSH (2.50 – 5.60 mU/L). The primary goal of this study was to determine whether women in the low-normal serum TSH group differed in markers of metabolism compared to women in the high-normal serum TSH group. The secondary goal of this study was to determine whether there was evidence of a linear association between TSH and measures of energy expenditure, macronutrient oxidation, and body composition.

The primary study hypothesis was rejected; mean energy expenditure, macronutrient oxidation rates and body composition variables were not significantly different between the low-normal and high-normal serum TSH concentration groups. Relationships between serum TSH concentrations and measurements of energy expenditure, macronutrient oxidation rates and body composition also were not significant when controlled for age, BMI, race, L-T4 treatment, menstrual stage, or when all factors were considered together. These findings are critical and provide evidence against redefining the upper limit of the normal serum TSH reference range based on differences in metabolic function.

Energy Expenditure and Serum TSH Concentrations

In a cross-sectional study of 104 overweight and obese, postmenopausal, euthyroid women, Rondeau et al. evaluated the relationship between total energy expenditure (TEE) by doubly labeled water (DLW) and plasma TSH concentration in the normal reference range divided into three groups (Group 1:0.34 - 1.66 mU/L, Group 2: 1.67 – 2.91 mU/L, Group 3: 2.94 – 4.84 mU/L). Total energy expenditure and plasma TSH concentrations were

negatively correlated, like our data, but were not significant (r = -0.141, p = 0.328). The results of our study are comparable to those reported by Rondeau et al. TEE measured by DLW was not significantly different (p = 0.09) between groups (low-normal: 2275 ± 413 kcals; high-normal: 2483 ± 485 kcals).

In contrast, Al-Adsani et al. investigated the effects of varying doses of levothyroxine (L-T4) on serum TSH concentrations and the effects on REE in nine subjects treated for hypothyroidism in a longitudinal study design. Each patient received three successive doses of L-T4, one to achieve normal TSH concentration, the second to achieve a slightly reduced TSH concentration and the third to achieve a slightly elevated serum TSH concentration over a 6-8 week period each. Both this study and ours targeted TSH concentrations within the normal reference range and compared the relationship to REE measured by indirect calorimetry. In their study, Al-Adsani et al. found that when higher doses of L-T4 were administered, serum TSH concentrations were lower and REE decreased by 15%, indicating sensitivity of REE to small changes in thyroid function (21). This study had a paired analysis, in which each participant acted as their own control over time. This method enhancece the ability to dete4ct differences in REE associated with small changes in TSH concentrations by limiting within-person variability between time points. Our cross-sectional study only evaluated the relationship of within normal reference range TSH concentrations and REE at one point in time. Unlike Al-Adsani, we found no significant linear association between normal reference range TSH concentrations and REE (p = 0.16).

Similar to our study, Boeving et al. measured the effects of serum TSH concentrations on resting energy expenditure (REE) in 42 patients treated with varying doses of L-T4 to achieve and sustain TSH concentrations within a low-normal TSH range (0.4-2.0 mIU/L, n=20) or a high-normal TSH range (2.0-4.0 mIU/L, n=22(20)). After the target TSH range was reached, patients were evaluated every 3 months for thyroid function, REE, body composition and bone mineral density for 12 months. There was a significantly

greater (p=0.02) relative increase in REE from the initial visit to the final visit in the low-normal TSH range group (7.1% ± 11.3%) compared to the high-normal TSH range group (3.6 % ± 15.1%) suggesting that treatment with L-T4 to achieve low-normal serum TSH concentrations was associated with an increased in REE (20). This study differs from our study in that it was longitudinal in design and their paired analysis showed change over time with L-T4 treatment. Our cross-sectional study, a less sensitive analytical model, detected no significant relationship in energy expenditure parameters and TSH concentrations across the normal reference range.

To our knowledge, our study reported here, is the first study to define the relationship of normal serum TSH concentrations and thermic effect of food (TEF). Our study did not demonstrate significant differences between low-normal and high-normal serum TSH concentration groups and TEF (mean difference of -2.85 kcal/meal, p = 0.50). In addition, no significant relationships were detected between serum TSH concentration and TEF (p = 0.67) alone or when corrected for age, BMI, race, L-T4 treatment, menstrual stage, and all factors considered together.

Macronutrient Oxidation and Serum TSH Concentrations

As thyroid hormones have been shown to have both hyper- and hypoglycemic effects, abnormal concentrations may influence metabolism by acting on lipogenesis. Because of these findings, we hypothesized that fat oxidation rates would be higher in women in the low-normal TSH group than women in the high-normal TSH group. Ortega et al. investigated associations of thyroid hormones and macronutrient oxidation in euthyroid, healthy Pima Indians. In this cross-sectional study, Ortega et al. reported that lower free T3 concentrations were an independent predictor of lipid oxidation. However, researchers did not find a significant relationship between TSH concentration and macronutrient oxidation (6). Our study did not measure the relationship between free T3 and macronutrient

oxidation. However, we did conclude that there was no significant association between higher fat oxidation rates and serum TSH as a measurement of thyroid function ($p = 0.22$).

Body Composition and Serum TSH Concentrations

Controversy continues within the field regarding the relationship between higher serum TSH concentrations and higher levels of adiposity. As several studies determined that there is a positive relationship between weight, body mass index (BMI) and serum TSH concentrations, we proposed that total mass, fat mass, and percent body fat would be significantly higher and lean body mass would be significantly lower in the high-normal serum TSH concentration group compared to the low-normal serum TSH concentration group.

Knudesen et al., reported a positive association between BMI and serum TSH concentrations ($P < 0.001$) and also showed that thyroid function was correlated to increased weight over 5 years. Researchers found a higher odds ratio for obesity with serum TSH above 3.6 mU/L, and a significant odds ratio of 2.1 was found when comparing serum TSH >3.6 mU/L to serum TSH of 1.0 – 1.99 mU/L (5). Svare et al., found that for each unit (mU/L) increase in TSH concentration among women, weight increased 0.9 kg and BMI increased 0.3 kg/m^2(30). Our findings reveal that weight ($p=0.05$) and BMI ($p=0.05$) are significantly different between the low-normal and high-normal TSH groups. The mean difference in weight between groups was -9.49 kg (95% CI: -19.1 – 0.1). The mean difference in BMI between groups was -3.43 kg/m2 (95% CI:-6.86 – 0.0). The relationship between weight and serum TSH concentrations became less significant when corrected for age, race, L-T4 treatment, menstrual stage and all factors considered. The relationship between BMI and serum TSH remained significant when correcting for age and race but became less significant when correcting for L-T4 treatment, menstrual stage and all factors considered.

Because we used a cross-sectional study design, we are unable to determine if higher weight causes higher serum TSH concentrations or if higher serum TSH concentration is related to higher weight. Total body mass, fat-mass, lean body mass and percent body mass were not significantly associated with serum TSH concentrations within the normal reference range.

Several studies suggest that the association between serum TSH and body weight may be influenced by hormonal signals from adipose tissue such as leptin (5). Our study did not measure leptin or any of the other putative adipokines to determine if varying concentrations of these hormones affected regulation of thyroid hormones.

Strengths of Study Design

We used a cross-sectional study design which allowed for us to compare many different variables at the same time. The major strength of this study is that gold standard measurements were used to assess each marker of metabolism. Measuring TEE by DLW provides an estimate of habitual TEE in unrestricted free-living individuals. Using indirect calorimetry to measure REE and macronutrient oxidation allows for accurate measurement of these components of energy expenditure. Body composition was measured by DEXA. The study used trained and licensed technicians to perform all measurements.

Another strength of this study was that women's menopausal status were recorded and used in the analysis. The menopausal status can significantly alter metabolism and affect circulating thyroid hormone concentrations. The physiology of perimenopausal women can differ significantly within and between pre- and postmenopausal women. Knowing this information helps strengthen our study by allowing for us to correct for hormone status in our analyses between low-normal and high-normal serum TSH groups.

Limitations

There are limitations to this research as well. The power of this cross-sectional analysis to detect differences in the outcome variables was not computed before the initiation of the study nor used to determine sample size. Our sample size was limited to the number of participants enrolled in the parent study who had completed all outcome variable measurements. However, we recognized this limitation, but also recognized the importance of carrying out this work to be able to establish the effect sizes for these variables for future powered analyses. The heterogeneity of our study may have impacted our ability to detect differences as well. The participants in this study varied by age, body weight and body composition. These differences may contribute to our observations that body weight and BMI were significantly different between low-normal and high-normal groups, but that no significant linear associations were seen when TSH was analyzed as a continuous variable.

Another limitation of this study was the accuracy of the 24-hour Urine Urea Nitrogen (UUN) measurement. The accuracy of the UUN measurement relies on the participant's ability to collect all urine excreted within a 24-hour period. 24-hour UUN requires study participants to carry around a container throughout the day to collect urine wghich is cumbersome and as a result, may lead to spilled samples or missed collections. Measurement of 24-hour urinary creatinine excretion may have been a useful method to validate completeness of the 24 hour total urine volume (39). UUN is an important determinant in calculating REE and macronutrient oxidation rates and calculations in this study may have been affected by the validity of incomplete 24 hour total urine volumes.

Also, our study sample is composed of predominantly Caucasian women. Therefore our findings may not be generalizable to other ethnic/racial groups. We also used a cross-sectional approach, which does not allow us to determine cause and effect between serum TSH concentrations and markers of energy expenditure, macronutrient oxidation and body composition. A longitudinal study design would have allowed for us to detect developments

in our target population at both the group and individual level. This type of study design would have allowed for us to study a sequence of events over time.

Future Research

Our study indicated that there were no significant associations between varying serum TSH concentrations within the normal reference range and markers of energy expenditure, macronutrient oxidation and body composition. However, some significant differences were seen between weight, BMI and low-normal and high-normal TSH concentrations. Further research in this area, including a longitudinal analysis with a larger high-normal TSH concentration sample size, should be conducted to better tease out the relationship between TSH concentrations and weight gain and other markers of metabolic function. It would be beneficial to examine these hypotheses among a more racially diverse sample, but one that has less variation in age and body composition parameters so that the results may have a broader application. Although the results of this study did not confirm our hypotheses and are not considered to be conclusive, this novel question merits further study.

Conclusion

Based on the cross-sectional nature of this study and the results reported here, we conclude that variations in TSH concentration within the established normal range are not associated with differences in body composition, energy expenditure or macronutrient oxidation parameters. We suggest that there is a positive relationship between TSH concentrations and increased weight and BMI. Further studies are needed to confirm the significance of these associations.

Appendix 1: Evidence-Based Table Summarizing the Impact of Thyroid Function on Energy Expenditure			
Study Citations	Study Design	# of Subjects/Characteristics	Outcomes
Reference range thyroid-stimulating hormone is associated with physical activity energy expenditure in overweight and obese postmenopausal women: a Montreal-Ottawa New Emerging Team Study. Rondeau G et al. Metabolism. 2010 Nov;59(11):1597-602. Epub 2010 Mar 31	Cross- Sectional Cohort	n=104 post-menopausal women, overweight/obese, spontaneously euthyroid women, ages 46-70, no use of hormone replacement therapy, sedentary Evaluated TEE (kcal/d) by DLW over a 10 day period Women were stratified into three groups by plasma TSH concentration: group 1(TSH 0.34-1.66 mU/L, n= 34), group 2 (TSH 1.67-2.91 mU/L, n=35), group 3 (TSH 2.04-4.84, n=35)	Total energy expenditure and plasma TSH concentrations were negatively correlated, but not statistically significant (r = -0.141, p = 0.328. Total energy expenditure did not vary significantly between the three groups.
Subclinical hypothyroidism in obese patients: Relation to resting energy expenditure, serum leptin, body composition, and lipid profile. Tagliaferri M, Berselli ME, Calò G, Minocci A, Savia G, Petroni ML, et al. Obes Res. 2001;9(3):196-201.		n= 108 obese patients (average BMI, 43.4 ± 6.6 kg/m^2) with subclinical hypothyroidism compared to a group of 131 obese (average BMI, 42.9 ± 6.8 kg/m^2) controls with normal serum TSH concentrations (2.1 ± 1.1 mU/L) matched for age, sex, and BMI A 7-day dietary record was obtained to assess daily energy intake (2843 ± 1386 kcal/24 h vs. control, 3148 ± 1551 kcal/24 h) Indirect calorimetry was performed to measure REE (1826 ± 362 kcal/24 h vs. control, 1821 ± 324 kcal/24 h)	None of these metabolic parameters were significantly different between the patients with subclinical hypothyroidism and the control. REE was indexed to FFM for comparison purposes. Patients were stratified into subgroups based on serum TSH concentrations to gain insight into the level of impairment of thyroid function that would result in differences in outcome variables. Sixty-three percent of the subclinical hypothyroid patients were assigned to the TSH <5.7 mU/L group had an estimated ~31 kcal/kg FFM and 37% that were assigned to the TSH >5.7 mU/L group had an estimated ~28 kcal/kg FFM (p<0.05). The authors concluded that subclinical hypothyroidism affected REE in obese patients when serum TSH concentrations were clearly above the normal range (>5.7 mU/L)

Low-normal or high-normal thyrotropin target levels during treatment of hypothyroidism: a prospective, comparative study. Boeving, A. Thyroid, Volume 21, Number 4, 2011	Prospective Study	N=42 newly diagnosed overt hypothyroid Participants were treated with varying doses of L-T4 to achieve and sustain TSH concentrations within a low-normal TSH range (0.4-2.0 mIU/L, n=20) or a high-normal TSH range (2.0-4.0 mIU/L, n=22) At target range subjects evaluated every 3 months for thyroid function and REE for 12 months. REE wsa measured with an indirect calorimeter	There was a significantly higher (p=0.02) relative increase in REE from the initial visit to the final visit in the low-normal range group (7.1% ± 11.3%) compared to the high-normal range group (3.6 % ± 15.1%) suggesting that treatment with L-T4 to achieve normal serum TSH concentrations was associated with an increased in REE
Resting energy expenditure is sensitive to small dose changes in patients on chronic thyroid hormone replacement. Al-Adsani H, Hoffer LJ, Silva JE. J Clin Endocrinol Metab. 1997;82(4):1118-25		n= 9 patients treated with L-T4 for hypothyroidism Each patient received three doses of L-T4, one to achieve normal TSH concentration, the second to achieve a slightly reduced TSH concentration and the third to achieve a slightly elevated serum TSH concentration over a 6-8 week period. Doses were increased or decreased between 25 and 50 µg/day based on the initial serum FT4 and TSH concentrations of the patient. Resting energy expenditure was measured by indirect calorimetry, fat-free mass (FFM) was measured by BIA and the ratio of REE/kg FFM was calculated for comparison purposes.	When increased doses of L-T4 were administered, serum TSH concentrations decreased in every patient by 0.17-18 mU/L and REE increased 5-10% in patients with TSH concentrations within the normal range. When L-T4 doses were decreased, serum TSH concentrations increased and REE decreased in every patient by 75-150 kcal/24 h Overall, when serum TSH concentrations increased between 0.1-10 mU/L, REE decreased by 15%, indicating sensitivity of REE to small changes in thyroid function

Muscle metabolism and exercise tolerance in subclinical hypothyroidism: A controlled trial of levothyroxine. Caraccio N, Natali A, Sironi A, Baldi S, Frascerra S, Dardano A, et al. J Clin Endocrinol Metab. 2005;90(7):4057-62.	Double-blind, randomized, placebo-controlled trial	n= 23 participants with subclinical hypothyroidism Each participant was treated 25 μg of L-T4 twice daily for 12 months. Participants were matched to 10 euthyroid controls (average TSH, 1.39 ± 0.18 mU/L) supplemented with a placebo for 6 months. Participants were involved in an incremental step-up cycling exercise protocol that measured oxygen consumed (VO_2), CO_2 produced (VCO_2), and heart rate. Blood glucose, lactate, pyruvate, free fatty acid, glycerol, and β-hydroxy-butyrate concentrations were measured before exercising, every 2 minutes during exercise, and during recovery. The exercise protocol was repeated after 6 months of placebo supplementation or 6 and 12 months of L-T4 treatment.	Despite treatment with L-T4 for 6 months, maximal power output (80 ± 8 watts, vs. control, 88 ± 13 watts, p=0.02) and maximal oxygen uptake (39.8 ± 2.4 mL/min/kg, vs. control, 41.9 ± 2.4 mL/min/kg, p=0.04) were lower in participants with subclinical hypothyroidism compared to euthyroid controls. During the exercise protocol, the respiratory quotient (RQ) increased in all participants suggesting that the rate of carbohydrate oxidation increased, and/or the rate of fat oxidation decreased, however the change in RQ from pre-exercise was significantly greater in the subclinical hypothyroid group (0.8 to 1.0) than in the controls (0.82 to 0.90, p<0.04). Despite these differences at 6 months, after 12 months of L-T4 treatment there was no significant changes in metabolic response to exercise among subclinical hypothyroid participants compared to baseline or compared to controls(22). Investigators showed that patients with subclinical hypothyroidism had an impaired metabolic response to exercise that was not corrected by restoring serum TSH concentrations to the normal reference range with L-T4 replacement over 6-12 months (22)

Substrate oxidation and thyroid hormone response to the introduction of a high fat diet in formerly obese women Buemann,B.,Toubro,S., Astrup,A. Int.J.Obes., 1998, 22, 9,869-877		n= 8 formerly obese women compared to 8 control women Energy expenditure and substrate oxidation rates were measured over 4 consecutive days while the women were housed in a whole-room calorimeter. On the first and fifth day of the study, each subject consumed a diet comprised of 30% of energy from fat, 55% from carbohydrate and 15% from protein. On the intervening days, subjects consumed a diet comprised of 55% of energy from fat, 30% of energy from carbohydrate and 15% from protein. Blood FT4 and FT3 concentrations were measured before the first high fat meal and again 3 days later.	Women in the obese group had a lower mean total energy expenditure (1981 ± 48 kcal/day) compared to women in the control group (2104 ± 58 kcal/day) while consuming the high fat diet, but the difference was not statistically significant ($p<0.13$). Plasma free T3/T4 ratio were lower in the formerly obese women after consuming a high fat diet (from 0.26 ± 0.02 to 0.19 ± 0.01 pmol/L, $p<0.005$) and positively associated with total energy expenditure such that lower T3/T4 ratios were associated with lower total energy expenditure. Plasma triglyceride concentrations were significantly lower in the obese group (0.93 ± 0.13 to 0.74 ± 0.07 mmol/L, $p<0.005$) than the control group (1.44 ± 0.21 to 1.06 ± 0.13 mmol/L) after consuming the high fat diet. There was a greater increase in RQ in the obese group (+0.053 ± 0.009) than the control group (+0.030 ± 0.005, $p=0.02$) after consuming the high fat diet suggesting a lower rate of fat oxidation

Appendix 2: Evidence-Based Table Summarizing the Impact of Thyroid Function on Body Composition			
Study Citations	Study Design	# of Subjects/Characteristics	Outcomes
Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population Knudsen,N., Laurberg,P., Rasmussen,L.B., Bülow,I., Perrild,H., Ovesen,L.,Jørgensen,T. J.Clin.Endocrinol.Metab., 2005, 90, 7, 4019-4024	Longitudinal	n= 4649 participants with previous or current overt thyroid dysfunction Participants were stratified into five subgroups based on serum TSH concentrations: group 1 (TSH <0.4 mU/L), group 2 (TSH 0.4 - 0.99 mU/L), group 3 (TSH 1.0 - 1.99 mU/L), group 4 (TSH 2.0 - 3.6 mU/L), and group 5 (TSH >3.6 mU/L)	At follow-up after 5 years, the difference in BMI between group 1 and group 5 was 1.9 kg/m^2 reflecting a difference in weight gain of 5.5 kg (5)
Serum TSH related to measures of body mass: Longitudinal data from the HUNT Study, Norway Svare,A.; Nilsen,T.I.L.; Bjøro,T.; Åsvold,B.O.; Langhammer,A. Clin.Endocrinol., 2011, 74, 6, 769-775	Longitudinal	n = 9954 women and 5066 men without self-reported thyroid disease and with serum TSH concentrations between 0.5-3.5 mU/L at baseline were studied Studied the relationship between serum TSH concentration and body composition and the associations between change in weight and change in serum TSH concentration after 10.5 years of follow-up	At follow-up, weight was 1.8 kg (95% CI 1.7, 1.9) higher and BMI was 1.0 kg/m^2 (95% CI 1.0, 1.1) higher in women than at baseline. Waist circumference and waist-to-hip ratio was also higher in women at follow-up by 10.5 cm (95% CI 0.9, 1.0) and 0.09 cm (95% CI 0.09, 0.09), respectively. Positive associations were seen between body composition measurements and serum TSH concentrations. As serum TSH concentrations increased by 1.0 mU/L, body weight increased by 0.9 kg, BMI increased by 0.3 kg/m^2, and waist circumference increased by 0.6 cm. A weight gain of 5 kg was associated with an increase in serum TSH of 0.08 mU/L. In contrast, serum TSH concentrations decreased by 0.12 mU/L among women whose weight decreased by 5 kg(30). This study concluded that weight gain is associated with increased serum TSH concentrations and weight loss is associated with decreased serum TSH concentrations

Plasma concentrations of free triiodothyronine predict weight change in euthyroid persons Ortega Martinez De Victoria,E.,Pannacciulli,N., Bogardus,C., Krakoff,J., Luther,S. Am.J.Clin.Nutr., 2007, 85, 2, 440-445	Longitudinal	n= 89 euthyroid adult Pima Indians Investigated the associations between thyroid hormone concentration and obesity.	Baseline FT3 concentrations were positively associated with absolute and annual percentage change in weight after 4 ± 2 years follow-up (p=0.02, p=0.009). Baseline plasma TSH concentrations were positively associated with body weight (p ≤0.01) and percent body fat (p<0.01), but not with FT3 or FT4 concentrations. The authors concluded that lower FT3 concentrations at baseline, but not FT4 concentrations, predicted weight gain.
Low-normal or high-normal thyrotropin target levels during treatment of hypothyroidism: A prospective, comparative study Boeving,A.; Paz-Filho,G.; Radominski,R.B.; Graf,H.; De Carvalho,G.A. Thyroid, 2011, 21, 4, 355-360	Prospective, interventional study	n= 42 patients with newly diagnosed overt hypothyroidism treated with L-T4 Stratified into two groups based on serum TSH concentrations: low-normal serum TSH (0.4 - 2.0 mU/L, n=20) and high-normal serum TSH (2.0 - 4.0 mU/L, n=22) Evaluated every 3 months for thyroid function, resting energy expenditure, body composition and bone mineral density over a 12 month period. Body composition was measured by DEXA and energy expenditure was measured by indirect calorimetry.(20)	At baseline the average BMI of the low-normal TSH group was 27 ± 5 kg/m^2 and the average serum TSH concentration was 58.9 ± 47.6 mU/L before treatment (20). The baseline BMI of the high-normal group was 29.3 ± 4 kg/m^2 and the average serum TSH concentration was 44.6 ± 26 mU/L. In the low-normal group, REE was 29.5 ± 2.9 kcal/kg-LM/day compared 30.4 ± 5.3 kcal/kg-LM/day in the high-normal group at baseline and was not statistically significant (p=0.5)(20). Lean mass was 36.3 ± 7.9 kg in the low-normal group compared to 38.5 ± 8.5 kg in the high-normal group at baseline. There were no differences in bone mineral density between groups. Total fat mass in both groups combined increased (from 25.9 ± 84.6 to 26.2 ± 95.5 kg, p=0.02) while lean mass decreased (from 42.3 ± 8.2 kg to 42.0 ± 7.3 kg, p=0.001) after 12 months of treatment. BMI and bone mineral density did not change or vary between groups

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